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ESTABLISHMENT STRATEGIES OF
SOME DECOMPOSER BASIDIOMYCETES

submitted by Christopher Dowson
for the degree of PhD
of the University of Bath

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SUMMARY

Somatic incompatibility systems of higher fungi, causing rejection of non-self, indicate that competition for spatial domain is a characteristic feature of fungal populations and communities. This study examines the capture and defence of domain by cord forming fungi, fairy ring fungi and an unusual wood decomposer Lenzites betulina.

L.betulina gains selective access to wood occupied by populations of Coriolus spp. by temporary parasitism of the resident mycelium after arrival by basidiospore. In contrast to this, non-unit restricted cord formers and fairy rings spread both sexually and vegetatively forming extensive individuals in woodland litter. Cord formers were easily introduced into a range of woodland sites via inoculated wood blocks from which initial outgrowth was dependant upon microenvironmental conditions, wheras establishment varied with litter type. Outgrowth of cords in the field and in soil tubes was usually several fold faster than mycelia on agar media. Mycelial interactions in soil, wood and malt agar revealed a combative heirarchy headed by species of Phanerochaete, with cord formers in general being more aggresive than other wood decomposers. However, the outcome of interactions was also influenced by inoculum size and substrate. In contrast to growth on agar, cord formers growing between discontinuous resources in soil exhibited coordinated regression and outgrowth of mycelia. These responses were strongly influenced by resource availability and the growth pattern of different fungi reflected varying foraging strategies. Fairy rings of Clitocybe nebularis were composed of polarised mycelial annuli which were differentiated into three distinct zones. Disruption of polarity, by loss of substrate or

interaction with other rings, resulted in lysis. Such responses were analogous to those described above with cord forming fungi. The underlying genetic interactions and developmental versatility during mycelial establishment are discussed.

CHAPTER 1.

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Temporary parasitism of *Coriolus* spp. by *Lenzites betulina*: a strategy for domain capture in wood decay fungi

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Key words: *Lenzites betulina*; *Coriolus* sp.; Parasitism; Wood decay

1. SUMMARY

Temporary parasitism by individuals of the wood-decaying basidiomycete *Lenzites betulina* allows them to gain selective access to wood occupied by populations of pioneer basidiomycete colonisers in the genus *Coriolus*. *Pseudotrametes gibbosa* is similarly temporarily parasitic on species of *Bjerkandera*. This strategy facilitates possession by the parasites of a large mycelial domain, which in turn allows them to produce large sporophores with a high reproductive output. This behaviour strongly resembles that of strangler figs and temporary social parasitism in ants.

2. INTRODUCTION

Until recently the essentially territorial behaviour of higher fungi has been overlooked, due partly to the belief that intraspecific competition is prevented because mycelia fuse to form collective units via hyphal anastomosis [1]. However, the discovery of widespread somatic incompatibility systems causing rejection of non-self, following anastomosis between different genotypes, has helped to establish the fact that competition for spatial domain (defined here as territory or space containing nutrients within the immediate sphere of influence of the thallus) is a characteristic feature of fungal populations and communities [2]. Hence, strategies for capture and defence of domain may be expected to occur in fungi which parallel those of higher plants and animals [3].

It is emerging that populations of decay fungi in intact standing tree trunks tend to be composed of one or a few genotypes occupying extensive domains. This is in contrast with populations arising from spore colonisation in cut, felled, fallen or broken (henceforth termed 'detached') wood, where numerous individual genotypes often exist side by side in small domains [3–6]. This difference is probably due partly to fewer opportunities for colonisation in the selective environment of the standing tree. On the other hand, fungi which colonise detached wood predominantly via mycelial cords or rhizomorphs frequently occupy large volumes of wood and form extensive systems

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interconnecting woody resource units on the forest floor [6–8].

Interestingly, species of fungi involved in decay of intact standing trunks also tend to produce larger fruit-bodies than those colonising detached wood by means of spores. Such differences in fruit-body size are not unexpected, since reproductive output depends on supply of nutrient resources and hence on mycelial domain [2,9]. It may be, therefore, that bulky fruit bodies are positively selected where individuals habitually occupy large domains but have limited opportunities for colonisation, but negatively selected when they have small domains and less restricted colonisation opportunities.

L. betulina appears to be an exception to this generalisation in that it colonises detached angiospermous wood via basidiospores but has bulky fruit-bodies (1–5 × 2–8 × 0.3–2 cm) [10]. Such a large commitment of fungal biomass to reproduction must come from occupying large domains (I. Chapela, L. Boddy and A.D.M. Rayner, in preparation), but how can this be achieved? The answer might be expected to lie, at least in part, in its interactions with other wood decay fungi. We were prompted to investigate this possibility following a report that *L. betulina* shows a non-antagonistic interaction with *Coriolus zonatus* and *Coriolus versicolor* [11], which was surprising since it is doubtful whether non-antagonistic interactions between wood decay fungi can actually occur [12,13].

3. METHODS

3.1. Interactions on agar plates

Two isolates of *L. betulina* were obtained from naturally occurring fruit-bodies and paired at a distance of 3 cm against a range of other fungi on 2% malt agar (MA: 2% w/v spray malt, A. Munton and Fison; 1.5% (w/v) agar) in the centre of 9-cm triple-vented Petri dishes. The plates were incubated at 15, 20 and 25°C under normal atmospheric conditions. In those pairings involving *C. versicolor* and *C. zonatus* against *L. betulina*, additional experiments on MA at 25°C were incubated under a range of gaseous conditions: O_2 and CO_2 percentages by volume were set at 5, 30; 5, 60; 20, 60 and 20, 30 respectively, the remaining gas being N_2.

3.2. Interaction between small plugs of L. betulina *inoculated into established colonies of other fungi on agar plates*

The experiments described in the previous section represent the situation where well established mycelia interact. Since in nature spores of *L. betulina* probably colonise wood which often already contains well established individuals of other species, the following two experiments were set up to mimic this situation.

Inoculum plugs of 2, 4, 6 and 8 mm diameter × 5 mm deep were taken from 20-day old cultures of *L. betulina*. Four plugs from each size category were placed on the aerial mycelium of 20-day-old MA cultures of *C. versicolor, C. zonatus* and *Stereum hirsutum* at 1, 2, 3 and 4 cm from the edge of the fully colonised plates. Each size category was placed on a separate plate and incubated at 20°C.

3.3. Interactions in wood blocks

Pairings were made between *L. betulina* and *C. versicolor, Phanerochaete velutina, S. hirsutum* and *Vuilleminia comedens* in different sized beech (*Fagus sylvatica*) wood blocks at 15°C or 25°C. *L. betulina* inocula, as 1-cm^3 infected wood blocks, were placed in contact either with one 10-cm^3 block, or sandwiched between two 10-cm^3 blocks, of the test fungi. *L. betulina* was also paired against the *Coriolus* spp. in 10-cm^3 blocks. Pairings, made in triplicate, were incubated for 15 days in covered beakers at 15°C and 25°C, and isolations were then made by transferring small chips of wood to MA.

4. RESULTS

4.1. Interactions on malt agar plates

The outcome of the interactions were classed as 'deadlock', with neither fungus able to invade the other, or 'replacement' of one fungus by another (Fig. 1a, b), usually involving overgrowth, sometimes following lysis. Usually, qualitatively similar

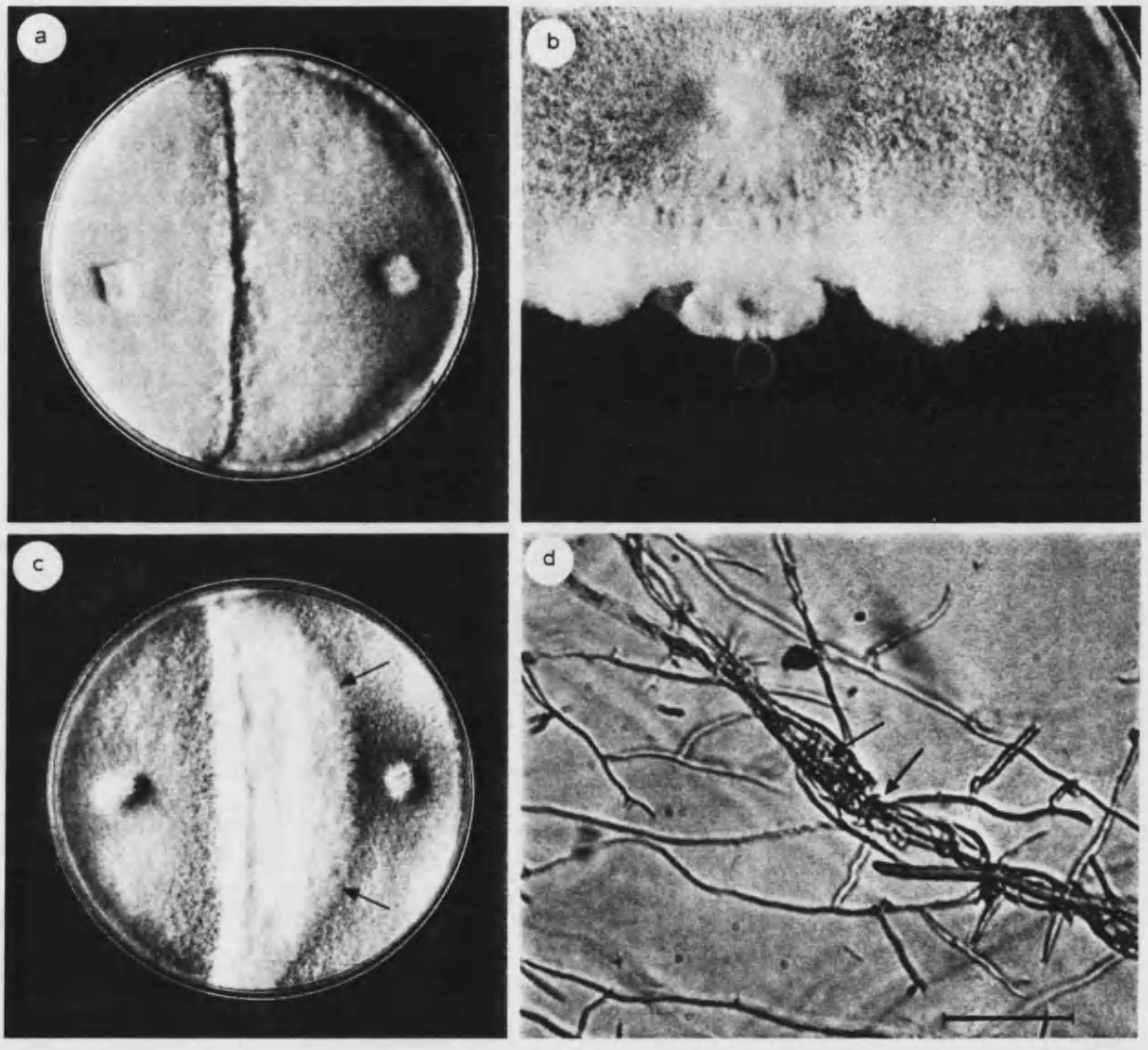

Fig. 1. Mycelial interactions on 2% malt agar in 7 cm petri dishes at 25°C. (a) Deadlock interaction between *Coriolus versicolor* and *Coriolus zonatus*; (b) replacement of *Phanerochaete velutina* (bottom) by *Lenzites betulina* (top); (c) interaction between *Lenzites betulina* (left) and *Coriolus zonatus* (right) showing development of luxurious replacement front (arrowed); (d) entwining of vacuolated hypha of *C. versicolor* (arrowed) by typical branches of *L. betulina*, scale bar 20 μm.

interactions occurred at each temperature, although occasionally the outcome was shifted from deadlock to replacement at 25°C (Table 1). Different isolates of *L. betulina* always produced the same outcome, but different isolates of *P. velutina* and *S. hirsutum* sometimes yielded different outcomes.

The interactions between *L. betulina* and the *Coriolus* species were particularly interesting. Direct through growth by *L. betulina* occurred against both *Coriolus* species, resulting in luxuriant growth of the former (Fig. 1c) without any inhibition of extension rate (2.5 mm/day). Subcultures taken from replacement fronts between *L. betulina* and

Table 1

Outcome of interactions between *L. betulina* and other wood decay fungi on 2% MA at different temperatures

D, deadlock; r, *L. betulina* replaced by other species; R, *L. betulina* replaced other species.

Wood decay species	15°C	20°C	25°C
Bjerkandera adusta	D	D	D
Coriolus versicolor	R	R	R
Coriolus zonatus	R	R	R
Heterobasidion annosum	r	r	r
Hypholoma fasciculare	D	D	r
Phanerochaete velutina (1)	D	D	D
(2)	R	R	R
Phlebia gigantea	r	r	r
Phlebia radiata	D	D	r
Phlebia rufa	D	D	D
Pseudotrametes gibbosa	D	D	D
Schizophyllum commune	D	D	D
Stereum hirsutum (1)	D	D	D
(2)	R	R	R
Vuilleminia comedens	D	R	R

Coriolus spp. yielded only the former species, even though mycelia of the latter can extend faster on agar. It seemed therefore that the *Coriolus* spp. were killed by *L. betulina.* This was confirmed by microscopic examination of interaction zones produced on low nutrient media (0.2% (w/v) malt), where vacuolated *Coriolus* hyphae were seen to be tightly entwined and penetrated by narrow branches of *L. betulina* (Fig. 1d).

The same reaction occurred under the whole range of temperature and gaseous regimes, although the progress of *L. betulina* through the *Coriolus* colonies was reduced under some of the non-atmospheric gaseous regimes. Thus, for both *Coriolus* spp. at 30% CO_2, 5% O_2 progress of *L. betulina* was significantly slower ($P \leqslant 0.05$; 1.5 mm/day) than at 30% CO_2, 20% O_2 or normal atmospheric conditions (2.5 mm/day). At 60% CO_2 and either 5 or 20% O_2, the extension rates of the *Coriolus* spp. were considerably reduced ($P \leqslant 0.05$), *C. versicolor* being more affected than *C. zonatus.* However, under these conditions the progress of *L. betulina* was not significantly different from that at 30% CO_2 ($P \leqslant 0.05$).

4.2. Interactions using small inocula of L. betulina *on agar plates*

The smallest inoculum plugs (2 mm diam.) of *L. betulina* were usually replaced when placed on mature colonies of either isolate of *S. hirsutum*, while the 4, 6 and 8 mm plugs usually showed deadlock or were occasionally replaced. Obvious appressed replacement zones were seen around all *L. betulina* inoculum plugs on *C. zonatus* and around all plugs from one of the *L. betulina* isolates on *C. versicolor.* With the other isolate of *L. betulina* on *C. versicolor* a deadlock response resulted.

4.3. Interactions in wood blocks

In the 10 : 1 and 20 : 1 v/v pairings, *L. betulina* was replaced by *P. velutina* and *V. comedens,* and exhibited a deadlock interaction with *S. hirsutum.* By contrast *C. versicolor* and *C. zonatus* were always replaced regardless of relative inoculum block size.

5. DISCUSSION

When *L. betulina* met most of the decay fungi against which it was paired, on agar or in wood, recognition was followed by deadlock or replacement of one species by the other. This outcome was sometimes modified under different abiotic regimes, as is often seen with wood decay species [14] (L. Boddy, unpublished). By contrast, in pairings between *L. betulina* and *C. versicolor* and *C. zonatus,* the last two species showed no signs of recognition of the former which replaced them by mycoparasitism under virtually all circumstances, even when the inoculum was very small. Clearly then, *L. betulina* is antagonistic to the *Coriolus* spp., and it was presumably the lack of recognition and associated typical reaction phenomena, such as production of pigmented zones, which led Henningson [11] to suggest otherwise.

Specific mycoparasitism of one wood decay fungus by another has also been detected with *Pseudotrametes gibbosa,* another fungus which produces large fruit bodies ($\leqslant 12 \times \leqslant 13 \times 1$–4 cm [10]) on detached wood [12]. Here *P. gibbosa* is mycoparasitic specifically on members of the

basidiomycete genus *Bjerkandera*. Similar reactions to those described between *L. betulina* and the *Coriolus* species were seen between *P. gibbosa* and *Bjerkandera adusta* or *Bjerkandera fumosa* on agar, and in paired wood blocks; moreover, cut sycamore stumps inoculated with *B. adusta* were selectively colonised by *P. gibbosa*.

B. adusta and *C. versicolor* are both very common dominant species at early stages of decay community development in detached angiospermous wood, where they often form populations of mutually antagonistic individuals occupying small domains. Although *B. adusta* and the *Coriolus* species may be found on wood of a wide variety of angiosperms, *B. adusta* and *P. gibbosa* occur especially on beech (*Fagus sylvatica*), whereas *C. versicolor, C. zonatus* and L. betulina favour birch (*Betula* spp.). One of the isolates of *L. betulina* we used came from a population on beech logs, but these had previously been extensively colonised by *C. versicolor* [5] (I. Chapela, L. Boddy and A.D.M. Rayner, in preparation). Both *B. adusta* and *C. versicolor* are very successful combatants against many other wood-rotting basidiomycetes.

We therefore propose that the ecological strategy of *P. gibbosa* and *L. betulina* is one in which, via temporary parasitism, they take over domain accessed initially by populations of *Bjerkandera* and *Coriolus*. Because *P. gibbosa* and *L. betulina* only become established relatively infrequently, large volumes of wood, which may previously have been held by large numbers of individuals of the host species, can become occupied by one or a few individuals of the parasites, enabling a large commitment of biomass to reproduction. Although the parasitism of one fungus by another (mycoparasitism) is a well-established phenomenon, it is usually viewed in terms of the host directly providing the parasite with a food source [15], rather than access to the domain in which a food source occurs.

Thus, the distinctive feature of the present phenomenon is that parasitism is incidental to competitive success, i.e. the acquirement of the domain occupied by pioneer populations. Although many fungi which become dominant late on in community development can gain access to such domain by non-selective antagonistic mechanisms, there is often an obvious recognition response by the incumbents and replacement is often a relatively slow process [2–8,13,14]. By contrast, temporary parasitism without a recognition response by the host allows less costly and more rapid replacement of specific pioneers, although the consequent selectivity reduces the overall opportunities for colonisation. Evidently, when *L. betulina* has become well established, it is able to replace some other species by more usual mechanisms, as indicated both in the present laboratory study and under field conditions (I. Chapela, L. Boddy and A.D.M. Rayner, in preparation).

A parallel strategy in higher plants is that shown by 'stranglers' in the genera *Ficus, Schefflera* and *Clusia* [16]. These germinate in the canopy of a host tree, then send down roots which, on reaching the soil, thicken, branch, anastomose and ultimately encase the trunk. The host tree is then overgrown, leaving the former epiphyte as an independent tree on its own roots. Another and even more striking parallel occurs in ants, which are temporarily socially parasitic [17]. Here the females cannot found colonies independently, instead invading those of other species, killing the host queen, and allowing their brood to be reared by host workers. The host colony gradually dies out whilst the number of social parasites increases until an independent colony emerges.

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CHAPTER 2.

New Phytol. (1988), **109**, 335–341

Inoculation of mycelial cord-forming basidiomycetes into woodland soil and litter

I. Initial establishment

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SUMMARY

Almost 1000 wood blocks, 8 cm^3, of beech (*Fagus sylvatica* L.), colonized by the mycelial cord-forming basidiomycetes *Hypholoma fasciculare* (Huds. ex Fr.) Kummer., *Phallus impudicus* (L.) Pers., *Phanerochaete* (*Ph.*) *laevis* (Fr.) Erikss & Ryv., *Ph. velutina* (DC ex Pers.) Parmasto, *Steccherinum fimbriatum* (Pers. ex Fr.) Erikss. and *Tricholomopsis platyphylla* (Pers. ex Fr.) Sing. were placed at the soil–litter interface in five different woodland sites. All species except *T. platyphylla* grew out radially from the blocks to form a network of mycelial cords ramifying amongst the litter and upper soil horizons. Initial outgrowth was more strongly related to microclimatic factors than to availability of nutrient resources, and after 3 months significantly greater ($P \leqslant 0{\cdot}05$) mean radial extension had occurred from blocks implanted during spring than from those implanted in winter or mid-autumn. Systems of *H. fasciculare* and *Ph. velutina* were always of significantly greater ($P \leqslant 0{\cdot}05$) radius than those of the other species after 3 and 6 months.

Key words: Biological control, fungal communities, mycelial cords, soil inoculation, wood decay.

INTRODUCTION

The successful introduction of a non-resident micro-organism into soil systems has usually been regarded as fraught with difficulty due to the 'biological buffering' effect of resident micro-organisms (Garrett, 1970; Corke & Rishbeth, 1980; Deacon, 1983). This particularly applies to organisms which can only establish themselves in the absence of competitors and is of special relevance to the development of systems for biological control of soil-inhabiting pathogens of plants. In cases where the introduced organism relies largely or wholly on the target organism for its nutrition, a stable stage in the life cycle of the control organism is required if repeated application is to be avoided. Alternatively, where the introduced organism utilizes energy resources other than the target organism, establishment and maintenance of its population will depend on these resources being available in an uncolonized and renewable form. Such conditions are rarely met, and this has greatly limited the scope for development of biological control, an exception being the use of *Phlebia gigantea* (Fr.) Donk to control the conifer root pathogen, *Heterobasidion annosum* Bref. (Rishbeth, 1963). Here *P. gigantea* is introduced into a selective and initially competitor-free environment, the surfaces of freshly cut stumps.

These requirements for effective resting stages or supply of competitor-free resources may, however, be obviated by introducing organisms which, as part of their life strategy, habitually establish themselves in stable competitor-filled environments by effective combat and replacement of residents. An example is provided by certain wood- and litter-decomposing basidiomycetes which form extensive systems of mycelial cords ramifying amongst the litter and upper soil horizons of the woodland floor (Thompson, 1984). Laboratory experiments have demonstrated the ability of these fungi to grow out from wood block inocula into non-sterile soil (Thompson & Rayner, 1982, 1983; Dowson, Rayner & Boddy, 1986; Dowson, Boddy & Rayner, 1988) whilst field

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observations have suggested that they are effective natural competitors of root pathogens such as *Armillaria* species (Rayner, 1977; Rayner & Todd, 1979; Thompson & Boddy, 1983).

These cord-forming basidiomycetes therefore seem to be suitable candidates for successful introduction into woodland soil and litter for such purposes as biological control and manipulation of nutrient cycling. A crucial starting point for assessing this possibility would be to attempt direct inoculation of a range of species under a variety of field conditions to characterize parameters influencing establishment, growth and persistence of cord systems. Here we report on patterns of initial establishment following such inoculation at three different times of year, using strains of six different species at five distinctive woodland sites.

MATERIALS AND METHODS

Strains and culture media

A single strain was selected to represent each of six cord-forming species. Strains of *Phanerochaete* (*Ph.*) *laevis* (Fr.) Erikss & Ryv. (Aphyllophorales), *Ph. velutina* (DC ex Pers.) Parmasto and *Steccherinum fimbriatum* (Pers. ex Fr.) Erikss. (Aphyllophorales) were derived from decayed wood of beech (*Fagus sylvatica* L.) sampled from Farleigh Hungerford Woods, Wilts (N. G. Ref. ST 795563). *Tricholomopsis platyphylla* (Pers. ex Fr.) Sing. (Agaricales) was isolated from decaying *F. sylvatica* wood from Savernake Forest, nr. Marlborough, Wilts. (N.G. Ref. SU 213682). *Hypholoma fasciculare* (Huds. ex Fr.) Kummer (Agaricales) was isolated from tissue of a fruit body produced on a log of *F. sylvatica* from Sallowvallets Inclosure, Forest of Dean, Gloucs (N.G. Ref. SO 611145). *Phallus impudicus* (L.) Pers. (Phallales) was isolated from a mycelial cord attached to a fruit body collected from Home Covert, Devizes, Wilts. (N.G. Ref. SU 008631).

All strains were routinely cultured on 2% (w/v) malt extract agar (MA: 20 g Munton & Fison spray malt A, 15 g Lab-M agar No. 2 per litre distilled water). Specimen cultures are being maintained at the School of Biological Sciences, University of Bath.

Preparation of wood block inocula

Wood blocks, approx. 8 $cm,^3$ were cut from freshly felled trees (approx. 15 cm diameter) of *F. sylvatica* from Colerne Woods, nr. Bath (N.G. Ref. ST 798725). The blocks were stored at −18 °C. Before use they were thawed, soaked in sterile distilled water for 3 h, and autoclaved in batches of 40 in foil-covered plastic beakers at 121 °C for 45 min. The blocks were placed onto 2-week-old cultures of *H. fasciculare*, *Phallus impudicus*, *Ph. laevis*, *Ph. velutina*, *S. fimbriatum* or *T. platyphylla* grown on 500 ml MA in 2 l wide-necked flasks. The flasks were incubated in darkness for 5 weeks at 20 °C before removal of the fully colonized wood blocks.

Field temperature

Temperature was monitored using maximum/minimum thermometers and chemical sensors. Thermometers were placed at the soil interface, and chemical sensors were placed both at the soil litter interface and within soil. The chemical sensors were based on the sucrose inversion technique which involves the acid-catalysed hydrolysis of sucrose to glucose and fructose, a reaction which results in a shift in the optical rotation of the solution from a positive to a negative value. At constant pH the rate of hydrolysis is dependent upon temperature, thus quantification of the change in polarization provides a method of estimating exponential mean temperature.

The procedure used was essentially similar to that adopted by Bocock, Bailey & Adamson (1977) (see also Berthet, 1960), but specific details and improvements were as follows. The reaction solution was buffered at pH 1·48 or pH 1·9 which allowed about 50% inversion during one month exposure in the winter or spring/autumn respectively. It was mixed in a conical flask on ice and 5 ml aliquots were then dispensed into plastic ampoules with screw tops (code 307, Sterilin Ltd, 43–45 Broad St, Teddington TW11 8OZ). The ampoules were either kept in ice and implanted in the field within 3 h or frozen for up to 28 days at −18 °C. Each time a set of ampoules was put out the reaction in one tube was stopped immediately, to act as a control, by adding either 4 μl or 20 μl of 6 M KOH to the pH 1·9 or pH 1·48 solutions respectively. After one month in the field reactions were stopped in the same way and tubes returned to the laboratory. (After adding akali the solutions were stable for at least 12 weeks at temperatures from 0 to 37 °C). Optical rotation was then measured with a Perkin-Elmer type 41 automatic polarimeter, using 1 ml of solution in a chamber of 1 cm path length. Experimental mean temperature was then calculated using the computer program of Boddy & Morris (1984).

Precipitation

An indication of the amount of precipitation was obtained every 2 months at several points on each site using 250 ml plastic conical flasks partially buried in the litter and soil, and fitted with 9 cm diameter funnels held in place by rubber bungs.

Matric potential

Soil moisture was recorded as matric potential using a modification of the filter paper technique simplified

Table 1. *Description of field sites*

Site	National Grid Reference	Soil type	Canopy composition and cover	Litter cover
A	ST 798723	Sandy loam	Pine (*Pinus nigra* Arnold) 100 %	2–3 cm
B	ST 797725	Clay loam	Beech (*Fagus sylvatica*) 80 % Larch (*Larix* × *eurolepis* Henry) 20 %	3–7 cm
C	ST 797727	Clay loam	Poplar (*Populus* sp.) 10 % Young spruce (*Picea abies* (L.) Karst.) 5 %	No litter (grass and moss)
D	ST 796728	Sandy clay	Larch 80 % Beech 20 %	3–4 cm
E	ST 796729	Clay	Oak (*Quercus robur* L.) 40 % Beech 30 % Cupressus sp. 20 %	1–5 cm

by Fawcett & Collis-George (1967). Whatman No. 42 filter papers (11·0 cm diameter) were washed in 0·005 % (w/v) mercuric chloride then dried at 105 °C. Three filter papers forming a sandwich were placed 1 cm below the soil–litter interface, with care being taken to replace soil and litter as found prior to implantation. After 2 weeks the centre filter paper was removed into a pre-weighed resealable 'Mini Grip' polythene bag. Each filter paper and bag was weighed to three decimal places and then dried at 60 °C to constant weight. Moisture content was expressed as per cent over dry weight and related to matric potential from the moisture characteristic curve of Whatman No. 42 filter paper (Fawcett & Collis-George, 1967).

Field sites and experimental design

Five sites, differing in tree species, canopy cover, litter composition and soil type, with few or no obvious mycelial cord systems present, were located at Colerne woods as shown in Table 1.

Wood block inocula, each colonized by one strain of *H. fasciculare*, *Phallus impudicus*, *Ph. laevis*, *Ph. velutina*, *S. fimbriatum* or *T. platyphylla*, were placed at the soil–litter interface of each site. Two inoculation arrangements were used. In the first (arrangement 1), a single species was introduced, placing one inoculum block at each of the four corners of a rectangle, 1·5 × 1·0 m. In the second arrangement (arrangement 2), six (or five when excluding *T. platyphylla*) species were inoculated in six (or five) columns and rows 14 cm by 17 cm apart in a randomized block design. Both types of inoculation arrangement were used in the experiment set up in December 1983, but only arrangement 2 was used when blocks were placed in the field during April and October. The arrays on individual sites were not replicated.

The initial outgrowth of mycelia from blocks in arrangement 2 was recorded 1, 2, 3 and 6 months from implantation, after excavation of the surrounding litter. Experiments using arrangement 1 were not excavated until after 6 months in order to assess the effects of litter disturbance on outgrowth.

RESULTS AND DISCUSSION

Soil temperature and moisture

Maximum intra-site variation of exponential mean temperature at any one period was only 0·8 °C, and

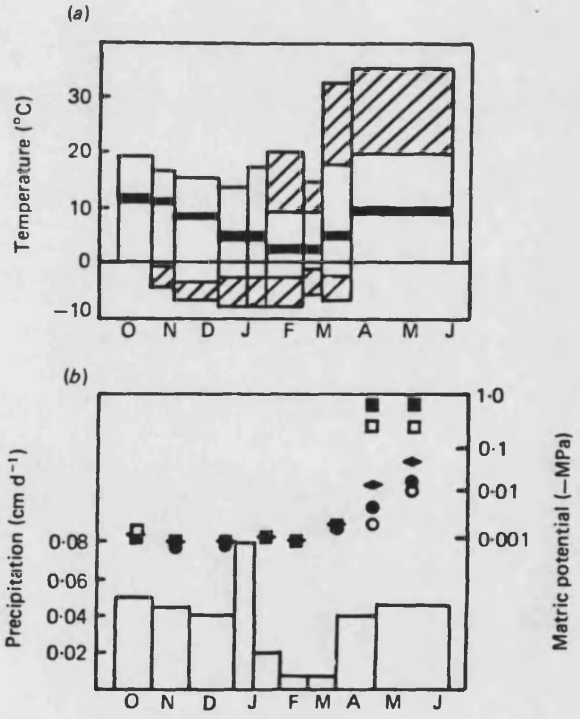


Figure 1. Microclimatic conditions at field sites from October to June. (*a*) Maximum and minimum temperatures at sites A, B, D, E (unhatched) and C, when significantly different ($P \leqslant 0{\cdot}05$) (hatched). Exponential mean temperature at the soil–litter interface for all sites (solid). (*b*) Mean precipitation for all sites and soil matric potential at Sites A (□), B (●), C (■), D (○) and E (◆).

intersite variation was 1·5 °C. For this reason the average value for all five sites is given in Figure 1. The most exposed site (C), having little tree cover, was subject to the most extreme maximum/minimum temperatures over this period, from 30 °C during spring to −8 °C during winter. For all other sites the range was from 20 to −5 °C (Fig. 1).

Little variation in soil moisture was detected within sites (≤ 5·0 % matric potential), but substantial variation was often found between sites even when rainfall was similar (Fig. 1). Rainfall at any one time was greatest at site C. Variation in matric potential therefore appeared to be primarily a function of soil composition and exposure due to canopy cover and litter depth.

Distribution and form of mycelium

Patterns of outgrowth of mycelia from inoculum blocks were seen to vary both between sites and between species. Tufts of mycelium were usually formed on the vertical faces of inoculum blocks 2 weeks after implantation, with the number of tufts appearing to decrease with an increase in the clay content of the surrounding soil. Where blocks were implanted in clay, tufts were only formed at the soil–litter interface and not below the soil surface.

Although most cords traversed the soil–litter interface some were produced deeper in soil. Their vertical distribution varied with soil composition, usually being no deeper than 3 cm in a sandy loam, 1 cm in a clay loam and 0·5 cm in a clay soil, although greater depths were attained, to a maximum of 7 cm, by cords growing along shrinkage cracks or rodent burrows. Mature cords found in the latter situations usually reappeared at the interface some distance from the point of initial entry. Cords were rarely found growing over exposed soil without some litter cover or close vegetation such as moss or grass. Beech leaves, when present as 50 % dry weight of the

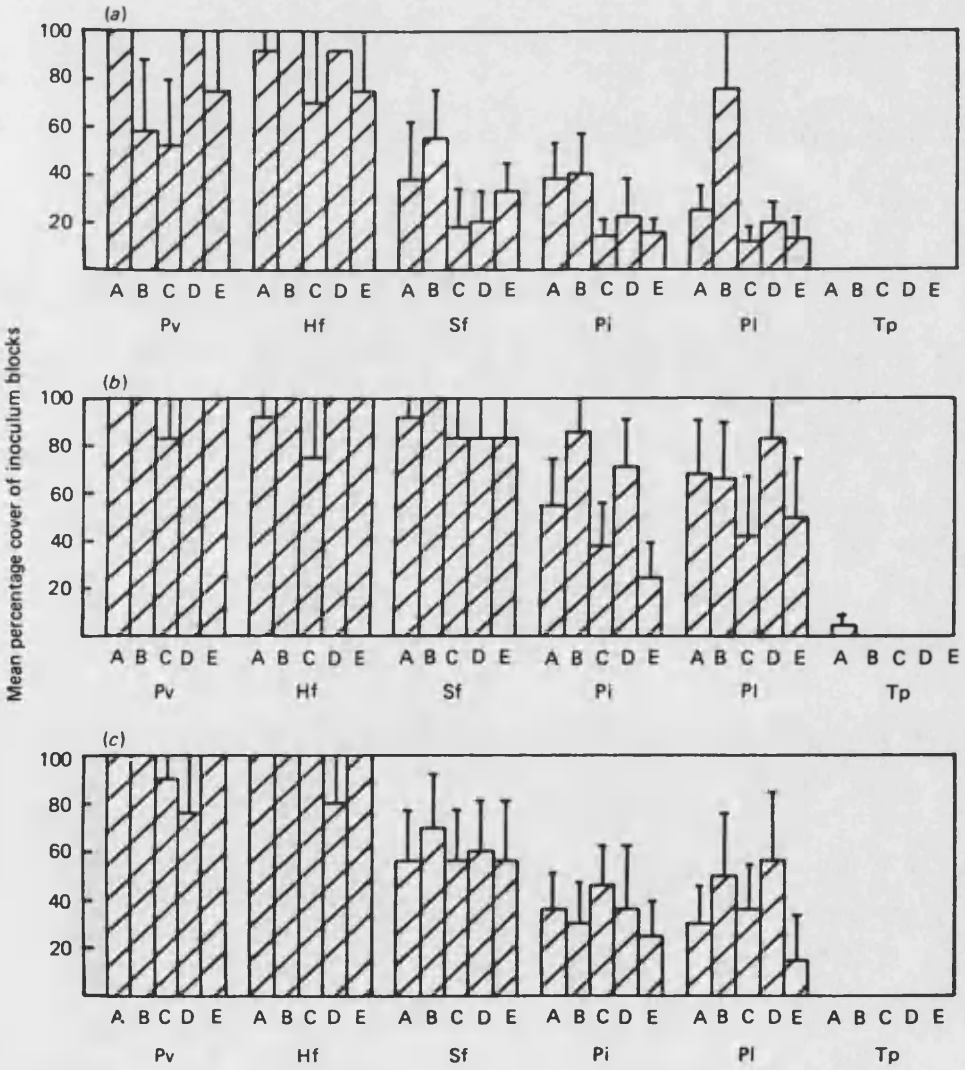


Figure 2. Percentage mycelial cover of inoculum blocks 3 or 6 months after implantation at sites A–E in winter, spring or autumn: (*a*) December to June. (*b*) April to June. (*c*) October to December. *Hypholoma fasciculare* (Hf), *Phallus impudicus* (Pi), *Phanerochaete laevis* (Pl), *Phanerochaete velutina* (Pv), *Steccherinum fimbriatum* (Sf) and *Tricholomopsis platyphylla* (Tp). Confidence intervals of 95 % indicated.

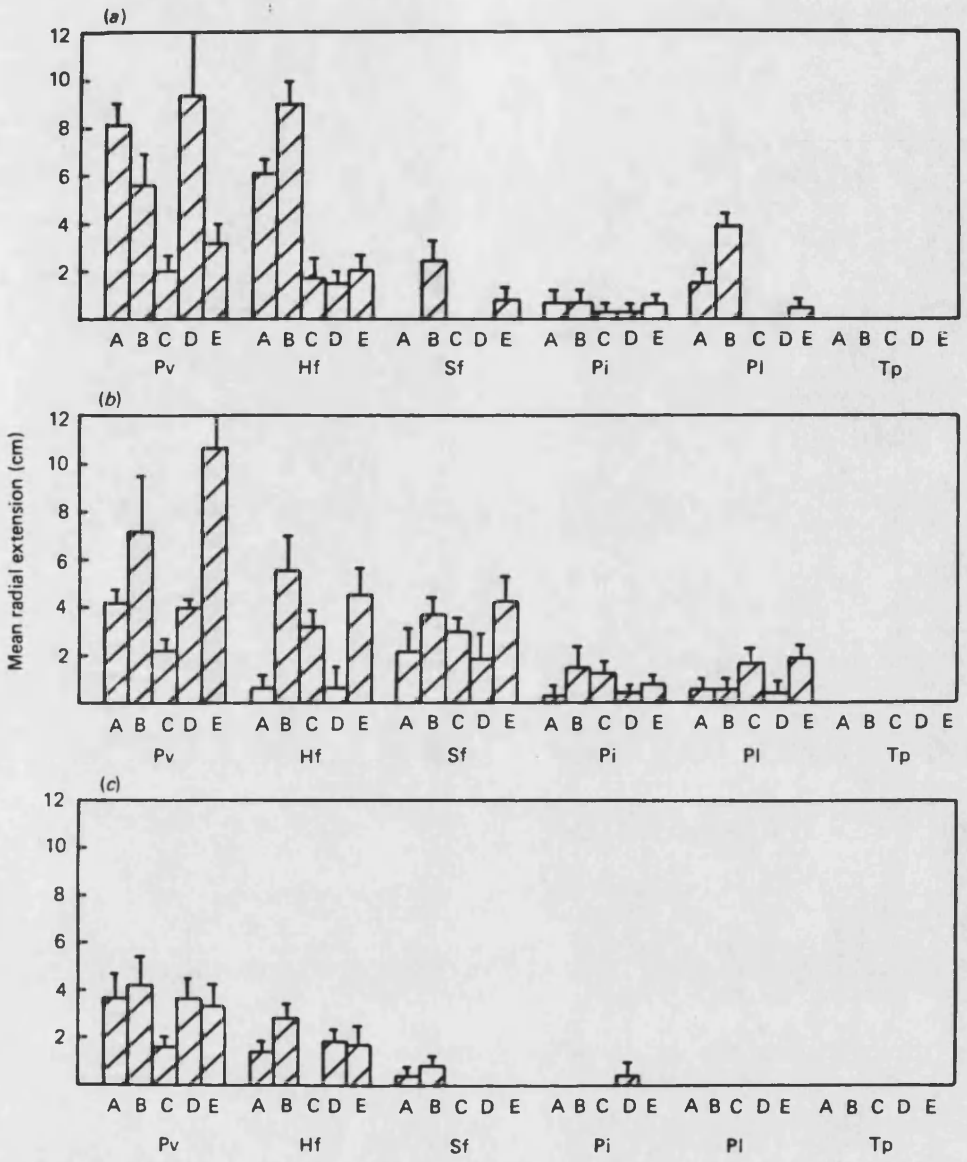


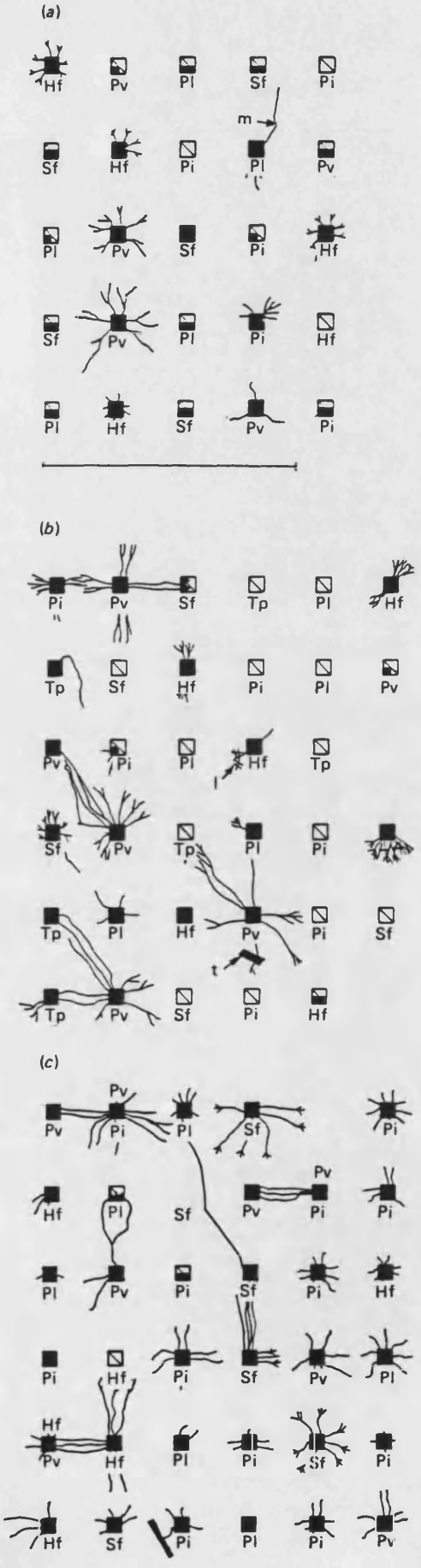
Figure 3. Radial extension of mycelial cords from wood blocks 3 or 6 months after implanting at field sites A–E during winter, spring and autumn: (*a*) December to June. (*b*) April to June. (*c*) October to December. Abbreviations as in Fig. 2. Confidence intervals of 95 % indicated.

total litter content, provided good protection both from freezing and desiccation, producing a layer 0·5–1·0 cm thick below which viable cords were readily formed. Exposed cords in such conditions soon died. A similar though more friable layer was formed by larch (*Larix* × *eurolepis*) needles, while pine (*Pinus nigra*) needles formed only a loosely woven mat offering still less protection. Cords only developed significantly in the uppermost litter layer during wet periods in autumn and spring.

The pattern of outgrowth of cords through litter varied. *S. fimbriatum* and *Ph. laevis* formed even, radiating systems of highly branched cords with a fan-like leading edge of finely divided cords. A similar pattern occured in *H. fasciculare*, but in some instances cords of this species were formed preferentially at inoculum block corners. Cord systems produced by *Ph. velutina* and *Phallus impudicus* tended to be more irregular. The strain of *T. platyphylla* used in these experiments failed to produce outgrowth. Few, if any litter components were obviously colonized by cords after 3 or 6 months, but extensive colonization did occur later (see Dowson, Rayner & Boddy, 1988).

Survival and outgrowth of mycelia from inoculum blocks

The vigour of each strain implanted using arrangement 2 during autumn, winter and spring is summarized both as the mean per cent mycelial cover of six inoculum blocks at each site (Fig. 2), and as the mean radial outgrowth into the surrounding soil and litter (Fig. 3).



Maximum outgrowth and mycelial cover occurred during spring, both for blocks implanted in April and scored in June ($3{\cdot}5 \pm 0{\cdot}5$ cm; 66 ± 11 %) and for those implanted in December which were still viable in April and scored in June. In the former case, outgrowth was significantly greater ($P \leqslant 0{\cdot}05$; *t* test) than in either the 3 month period from October to December ($0{\cdot}9 \pm 0{\cdot}4$ cm; 42 ± 11 %) or from December to March ($0{\cdot}1 \pm 0{\cdot}2$ cm; 44 ± 11 %). In the case of those implanted in December, despite having grown for 3 months longer, neither radial extension nor mycelial cover ($2{\cdot}2 \pm 0{\cdot}9$ cm; 54 ± 11 %) were significantly different ($P > 0{\cdot}05$) from those following inoculation in April. The blocks implanted in December gave lower mean values due to non-viable inocula being included in the calculations. In terms of vigour, whether recorded as mycelial cover or radial extension, the six species could be ranked in the order *Ph. velutina* = *H. fasciculare* > *S. fimbriatum* > *Phallus impudicus* = *Ph. laevis* > *T. platyphylla*. *Ph. velutina* and *H. fasciculare* in particular usually exhibited both a significantly higher percentage mycelial cover and greater radial extension ($P \leqslant 0{\cdot}05$; *t* test) than the other species. On the other hand, the performance of *T. platyphylla* was always significantly lower ($P \leqslant 0{\cdot}05$; *t* test) than that of the other species. Mean per cent mycelial cover and radial extension after 6 months for all species varied from 49 ± 11 % mycelial cover, $0{\cdot}7 \pm 0{\cdot}9$ cm radial extension at site C to $6{\cdot}7 \pm 11$ %, $3{\cdot}0 \pm 3{\cdot}2$ cm at site B, but were not statistically significantly different ($P \leqslant 0{\cdot}05$; *t* test). However, with the exception of *T. platyphylla*, significant intersite differences in radial extension (but not mycelial cover) were detected for individual species. These intersite differences in radial extension varied according to the time of inoculation. For example, on the basis of records made in June, extension of *Ph. velutina* was maximal at Sites A ($8{\cdot}2 \pm 0{\cdot}8$ cm) and D ($9{\cdot}4 \pm 2{\cdot}6$ cm) when implanted in April (Fig. 3). Extension of *P. velutina* at site C (the exposed site) was significantly less ($P \leqslant 0{\cdot}05$) than that at the other sites for all periods of the year. *Ph. laevis* behaved similarly, growing most rapidly at Site B from December to June but at Sites C and E from April to June. Conversely, *H. fasciculare* always extended maximally at Site B, and the relative growth at sites A and E altered with season.

In general, no significant differences ($P \leqslant 0{\cdot}05$; *t* test) in radial extension after 6 months were detected between the experiment which had been left un-

Figure 4. Outgrowth patterns at Site D. (*a*) 3 months after inoculation in October; (*b*) 6 months after inoculation in December; (*c*) 3 months after inoculation in April. The percentage mycelial cover of blocks is represented for 100% (■), 50% (⬓), 25% (⬔) and 0% (▨). Abbreviations as in Fig. 2. Superscript indicates replacement of resident species. Mycelial cord (m), *Larix* needles (l), twig (t). Scale bar 0·5 m.

disturbed (arrangement 1), and the experiments excavated after 1, 2, 3 and 6 months (arrangement 2). However, extension of *Ph. laevis* was significantly lower ($P \leqslant 0{\cdot}05$) in the unexcavated sites, 0·2 cm, as compared to the excavated sites, 1·6 cm.

Outgrowth patterns in arrangement 2 experiments at Site D after either 3 or 6 months from inoculation are broadly representative of patterns at the other sites (Fig. 4). In some cases interconnections between inoculum blocks had been established, occasionally associated with replacement of the former resident.

CONCLUSIONS

These observations of early stages of outgrowth following inoculation clearly demonstrate the ability of several cord-forming basidiomycetes to establish themselves in soil under field conditions. At this stage outgrowth is predominantly exploratory and dependent on nutrient resources supplied from the inoculum base. Consequently it is radially symmetrical, and governed by microclimatic factors rather than availability of resources for colonization. By contrast, later stages of development and persistence are strongly affected by resource availability and the combative ability of the fungi to defend their domain or wrest it from others. These themes are taken up in the second paper in this series (Dowson, Rayner & Boddy, 1988).

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Inoculation of mycelial cord-forming basidiomycetes into woodland soil and litter

II. Resource capture and persistence

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SUMMARY

Substantial mycelial cord systems of *Hypholoma fasciculare* (Huds. ex Fr.) Kummer, *Phallus impudicus* (L.) Pers., *Phanerochaete* (*Ph.*) *velutina* (DC ex Pers.) Parmasto, *Phanerochaete laevis* (Fr.) Erikss. & Ryv. and *Steccherinum fimbriatum* (Pers. ex Fr.) Erikss. had developed 2 years after direct inoculation into the soil and litter of a range of woodland sites. Where species had been inoculated individually, the distribution patterns of the cord systems were strongly related to the availability and colonizability of various litter components. Where different species were co-inoculated in columns and rows 14 × 17 cm apart, *Ph. velutina* frequently replaced the other species, forming extensive mycelial systems and bringing about considerable decay of the original inocula. However, established systems of *S. fimbriatum* often developed in close proximity to *Ph. velutina*. Where inoculum blocks were implanted amongst litter containing few available resources for colonization, outgrowth was strongly directed along rows of nearest-neighbouring blocks.

Key words: Biological control, fungal communities, fungal interactions, mycelial cords, soil inoculation.

INTRODUCTION

In a previous paper (Dowson, Rayner & Boddy, 1988*a*) we described early patterns of establishment of cord-forming basidiomycetes from wood blocks inoculated directly into the soil and litter of a variety of coniferous and deciduous woodland sites. At this early stage outgrowth patterns depended mainly upon microclimate and resources from within the inoculum block. However, successful introduction of cord-forming fungi depends not only on the initial outgrowth patterns, but also on the capacities for independent resource capture and competition with other organisms which underpin persistence and mobility on the woodland floor. Differences in these capacities between mycelial cord-forming species result from varying colonisation strategies, and will affect the choice of strains for successful introduction for such purposes as biological control and manipulation of nutrient cycles. Such differences can only be ascertained experimentally from long-term studies; here we report on results obtained up to 2 years after inoculation.

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MATERIALS AND METHODS

Details of the field sites, methods of preparation and introduction of inocula, environmental monitoring and excavation and mapping of mycelial cord systems are given by Dowson, Rayner & Boddy (1988*a*). Basically, six fungi, *Hypholoma fasciculare* (Huds. ex Fr.) Kummer, *Phallus impudicus* (L.) Pers., *Phanerochaete* (*Ph.*) *laevis* (Fr.) Erikss. & Ryv., *Phanerochaete velutina* (DC ex Pers.) Parmasto, *Steccherinum fimbriatum* (Pers. ex Fr.) Erikss. and *Tricholomopsis platyphylla* (Pers. ex Fr.) Sing., growing on wood block inocula, were implanted at the soil–litter interface, at five woodland sites, in two experimental arrangements, at different times of the year. In arrangement 1, four inoculum blocks of the same species were placed at each corner of a 1·5 × 1·0 m rectangle. In arrangement 2, inocula of all

Table 1. *Litter components colonized by cord-forming fungi 2 years after implantation at five different woodland sites*

Site and canopy trees	A *Pinus nigra*	B *Fagus sylvatica* *Larix* × *eurolepis*	C *Populus* sp. *Picea abies*	D *Larix* × *eurolepis* *Fagus sylvatica*	E *Quercus robur* *Fagus sylvatica* *Cupressus* sp.
Hypholoma fasciculare	None	*Fagus* twigs leaves, cupules	None	None	None
Phallus impudicus	None	*Fagus* twigs	None	None	None
Phanerochaete laevis	None	*Fagus* twigs leaves	None	None	None
Phanerochaete velutina	Cones, scales, needles (at edge of system)	*Fagus* twigs	None	*Fagus* twigs	*Fagus* twigs *Quercus* twigs
Steccherinum fimbriatum	Cones, scales, twigs	*Fagus* twigs, leaves	None	None	*Quercus* twigs, *Fagus* twigs, leaves, *Cupressus* twigs
Tricholomopsis platyphylla	None	None	None	None	None

species were placed at 14 cm intervals (columns) in rows set 17 cm apart, the position of each species within a row being chosen randomly. After 2 years, in order to check whether the mycelial cord systems observed in the field were genetically the same as those originally inoculated, a system of testing for somatic incompatibility was used (cf. Rayner *et al.*, 1984). Isolates of cord-forming fungi were obtained by transfer of small wood chips, excised aseptically from the centre of wood blocks, twigs or cones, onto 2% (w/v) malt extract agar (MA) containing 100 ppm novobiocin. These isolates were then paired against the strains originally inoculated by placing mycelial plugs 4 cm apart on 2% MA and incubating at 15 °C for 5–15 weeks. Intermingling, indicative of self-fusion, or development of a demarcation zone due to non-self rejection (somatic incompatibility) was noted. The results described below concern only those systems confirmed by a self-fusion response to be of the same genetic origin as the inoculated strains.

RESULTS AND DISCUSSION

Colonization of litter components

During the first 6 months after introduction to the field, mycelial systems had not extended far and hence few litter components were reached and colonized: *Ph. velutina* and *H. fasciculare* encountered and colonized more litter components than most because of their superior extension rates at low temperatures (Dowson *et al.*, 1988*a*).

The range of components colonized by isolates at each site after 2 years is summarized in Table 1. *T. platyphylla* was absent from all inoculum blocks and had not managed to colonise any litter components. *S. fimbriatum* and *Ph. velutina* colonized the widest range of litter components including both angiospermous and coniferous types, whilst *Ph. laevis*, *H. fasciculare* and *Phallus impudicus* were restricted either to the twigs and leaves or only the twigs of *Fagus sylvatica* L. *Pinus nigra* Arnold cones, predominantly without scales, were commonly colonized by penetration at the abscised end and re-emergence at the opposite end. Large twigs were colonized subcortically either via broken ends or where bark was disrupted. Thin *Cupressus* L. twigs and *Pinus nigra* needles were colonized via external mycelial sheaths. The latter usually only occurred at the periphery of cord systems where there were finely divided cords. Needles of *Larix* × *eurolepis* Henry, found infrequently at site B but the major component at site D, were apparently uncolonized by any strains. No litter was present at site C.

Development of mycelial systems in arrangement 1 experiments

The sizes of systems attained after 2 years (Table 2) from groups of four blocks of the same strain (i.e. in the absence of inoculated competitors) were related to the resource quality and distribution of naturally occurring litter components, and probably also to microclimate (Table 1). Established systems of *H. fasciculare*, *Ph. laevis* and *Phallus impudicus*, 5·5, 13 and 6 m in length respectively, were found only at site B where the litter was deep (3–7 cm), and predominantly *F. sylvatica* (see Dowson *et al.* 1987*a*). However, *Ph. velutina* and *S. fimbriatum* were both able to establish at three sites; A, B and D, and A, B and E respectively. The largest systems were formed at site B by *Ph. laevis* and *Ph. velutina* (total length 13, 12 m respectively) and at site E by *S. fimbriatum* (14 m), both sites being abundantly supplied with angiosperm leaves and twigs (Table 1). Systems of *Ph. velutina* and *S. fimbriatum* were

Table 2. *Total length of cord systems developing from inoculum blocks and persistence of fungi in inoculum blocks two years after implanting in five woodland sites*

	Species inoculated and site																													
	Hypholoma fasciculare					*Phallus impudicus*					*Phanerochaete laevis*					*Phanerochaete velutina*					*Steccherinum fimbriatum*					*Tricholomopsis platyphylla*				
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Total length (m) in arrangement 1*	0	5·5	0	0	0	0	6·0	0	0	0	0	13·0	0	0	0	3·0	12·0	0	8·0	0	3·5	6·0	0	0	14·0	0	0	0	0	0
Total length (m) in arrangement 2	0	0·1	0	0	0·1	0	0	0	0	0	0	0·1	0	0	0	6·4	5·4	3·1	5·0	5·6	1·8	0·5	0	0	0·4	0	0	0	0	0
Blocks containing strain originally present (%)	0	29	0	44	19	0	9	0	0	8	0	20	0	31	12	0	7	0	40	37	36	9	0	0	38	0	0	0	0	0
Blocks colonized by an implanted strain (%)†	0	36	0	55	37	0	9	0	0	8	0	20	0	31	16	186	44	67	187	125	72	9	0	0	38	0	0	0	0	0
Blocks in which original strains were replaced by non-cord-forming fungi (%)	5	10	0	17	28	23	18	0	27	45	27	20	0	45	21	11	40	0	9	6	17	18	0	40	29	6	0	0	33	50
Blocks in which original strains were replaced by cord-forming fungi (%)	47	0	0	20	20	48	28	5	18	39	45	10	9	0	46	0	0	0	0	0	35	28	9	40	42	52	0	0	17	0
Blocks in which original strains had been replaced and blocks almost completely decayed (%)	3	57	100	14	33	29	45	95	25	8	28	50	91	23	21	0	0	0	0	0	23	45	84	20	22	42	100	100	50	50
Blocks almost completely decayed by strain originally present (%)	10	5	0	5	0	0	0	0	0	0	0	0	0	0	0	89	53	100	51	57	0	0	0	0	0	0	0	0	0	0

* Arrangement 1 layouts consisted of four inoculum blocks of the same species placed at each corner of a 1·5 × 1·0 m rectangle. All other data were obtained from arrangement 2 layouts of inocula of different species placed at 14 cm intervals in rows set 17 cm apart, the position of each species within a row being chosen randomly.

† Per cent of original number of blocks implanted.

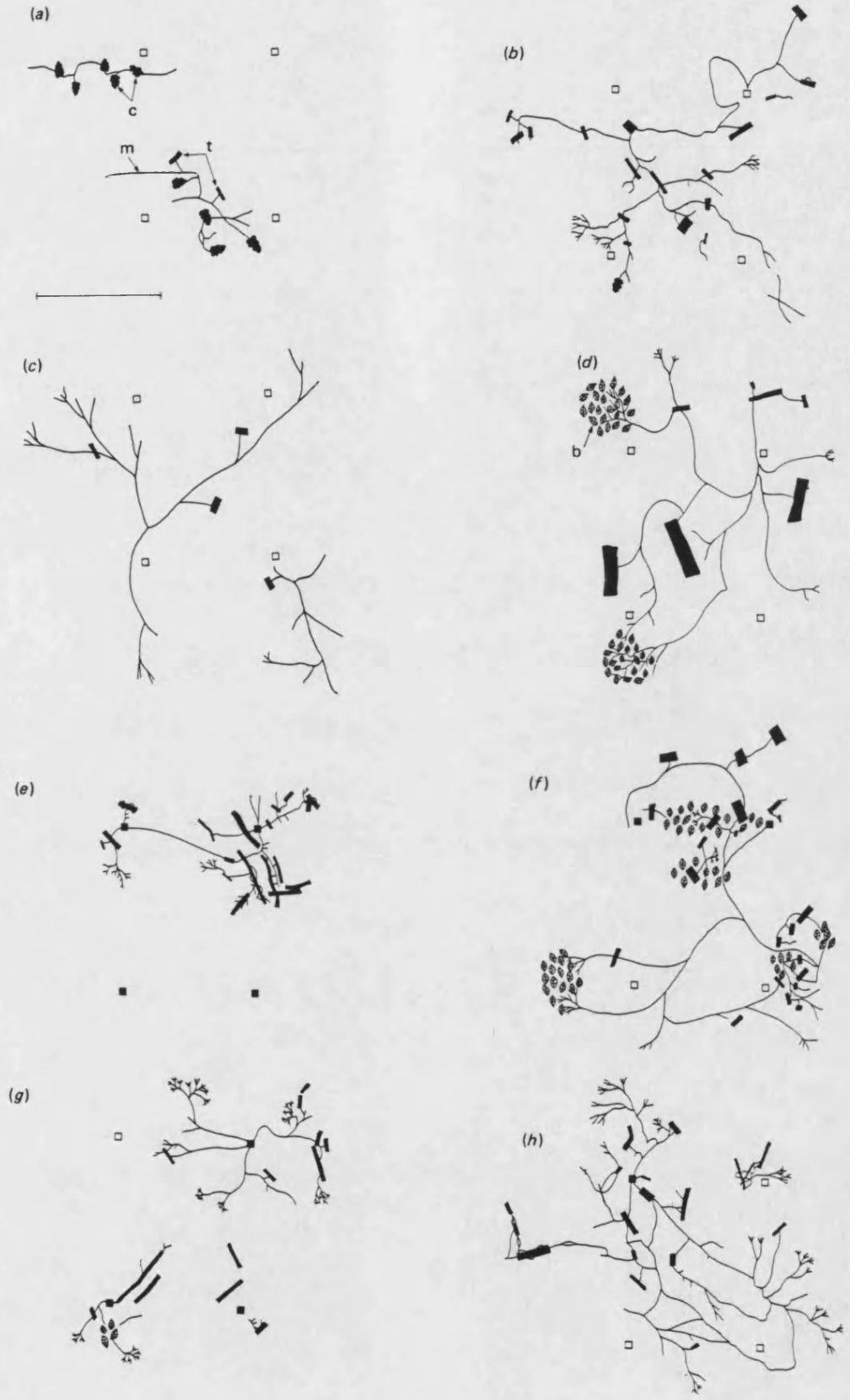

Figure 1. Development patterns of single mycelial systems (arrangement 1) in relation to resource type and distribution. (*a–c*) *Phanerochaete velutina* at sites A, B and D respectively. (*d–g*) *Hypholoma fasciculare*, *Phallus impudicus*, *Phanerochaete laevis* and *Steccherinum fimbriatum* respectively at site B. (*h*) *S. fimbriatum* at site E. (■), Inoculum block; (□), inoculum block decayed or absent. Mycelial cord (m), *Pinus* cone (c), twig (t), *Fagus* leaves (b) Scale bar represents 1 m.

respectively only 3.0 and 3.5 m long at site A, indicating that *Pinus* litter components provided a less suitable habitat than *F. sylvatica* or mixtures of *F. sylvatica*, *Quercus robur* L. and *Cupressus*. Similarly, *Larix* litter did not appear to support much mycelial extension because *P. velutina* was the only species to produce a system of significant length (8 m) at site D, this being due to colonization of *F. sylvatica* twigs.

Development patterns varied both with species and resource distribution. This can be seen, for example, by comparing systems of *Ph. velutina* at sites A, B and D [Figs 1 (*a–c*), 2], and from the range of patterns produced by *H. fasciculare*, *Phallus impudicus*, *Ph. laevis* and *S. fimbriatum* at site B [Fig. 1 (*d–g*)]. The simple radial growth pattern of *S. fimbriatum* at sites A and B (Fig. 1 *g*) constrasted with that at site E (Fig. 1 *h*), where during the same period of time a mature system of apparently stable interconnecting loops had developed some distance from the main group of colonized resources.

Development of mycelial systems in arrangement 2 experiments.

After 2 years, the presence of *H. fasciculare*, *Phallus impudicus*, *Ph. laevis*, *P. velutina*, *S. fimbriatum* and *T. platyphylla* within 36 inoculum blocks in arrangement 2 experiments differed radically from that at implantation (Fig. 2, Table 2). Moreover, the development of systems within these arrays contrasted markedly with those in arrangement 1 experiments (Fig. 1, Table 2).

T. platyphylla was completely lost at all sites, and *Phallus impudicus* was almost eliminated. Less dramatic changes were seen for other species and fewest changes in species present in inoculum blocks were detected at sites D, A and E. At site D between 31 and 44% of the original inocula of *Ph. velutina*, *H. fasciculare* and *Ph. laevis* remained, whilst at sites A and E, 36 and 38% of original *S. fimbriatum* inocula remained respectively. The main reasons for loss of original species from inoculum blocks were complete decomposition of the blocks, replacement by co-inoculated species, particularly *Ph. velutina*, and replacement by non-inoculated fungi. The latter were predominantly Hyphomycetes, in particular *Trichoderma* Pers. ex Fr. spp. and *Penicillium thomii* Maire. More occasionally found were the Ascomycotina *Ascocoryne sarcoides* (Jacq. ex S. F. Gray) Groves and Wilson, *Hypoxylon serpens* (Pers. ex Fr.) Fr. and *Xylaria hypoxylon* (L. ex Hooker) Greville; the Basidiomycotina *Coprinus domesticus* (Bolt ex Fr.) S. F. Gray (*Ozonium* stage) and *Sisrotrema brinkmannii* (Bres.) Erikss.; the Hyphomycete *Botrytis cinerea* Pers. ex Per., and various mucoraceous fungi.

Reasons for the loss of originally inoculated strains varied between sites. Maximum replacement and decay occurred at the exposed site (C), whilst maximum replacement by non-inoculated fungi took place at sites D and E. Colonization and replacement by cord-forming fungi resulted when radially extending systems encountered inoculum blocks no longer colonised by viable mycelia, inoculum blocks colonized by viable mycelia, litter components, or cords of other species. During initial outgrowth (see Dowson *et al.*, 1987*a*) *Ph. velutina* and *H. fasciculare* exhibited the greatest radial extension and therefore reached locally available resources before other inoculated species. After 2 years *P. velutina* had also colonized inoculum blocks and litter components initially occupied by *H. fasciculare*, *Phallus impudicus*, *Ph. laevis* and often also those occupied by *S. fimbriatum*.

Mycelial cord systems developing in arrangement 2 experiments were usually significantly shorter ($P \leqslant 0{\cdot}05$; t test) than those arising in arrangement 1 experiments (Table 2). However, at sites A, C and E, where *Ph. velutina* was restricted in outgrowth in arrangement 1 experiments by lack of available resources, extension was facilitated in arrangement 2 experiments by colonization of other inocula. This is evident in Table 2 from the high number of inocula colonized by *Ph. velutina* at these sites and the increase in maximum system length from 3·0 to 6·4, 0·0 to 3·1 and 0·0 to 5·6 m, in arrangements 1 and 2 respectively. Such results accord with the limitation of spread of most species by confrontation with neighbours, but the enhancement of spread of *Ph. velutina* owing to its capacity to replace neighbours.

Although it was clear from arrangement 1 experiments that *Ph. velutina* could extend for distances greater than those between the blocks, in arrangement 2 experiments on site A (where resources were sparse) it was observed that mycelia tended to colonize only the nearest block (14 cm away). This resulted in extension of mycelial growth along parallel rows [Fig. 2 (*a*,*b*)]. An explanation for this comes from observations by Dowson, Rayner and Boddy (1986) showing that colonization of a resource at one point on the periphery of a radially extending system resulted in the rapid cessation of extension of non-connective mycelium followed by regression, with outgrowth from the newly colonized resource maintaining its original polarity. Such a 'stop-go' pattern of growth would result in the observed colonization only of nearest neighbours and may also contribute to the reduction of overall length of systems in arrangement 2 experiments compared with arrangement 1.

Contact between rows did occur, however, and may have been mediated by several mechanisms. These include fusion of two approaching fronts from adjacent rows which spanned the required gap, lack of regression of some cords, colonization of litter components between rows which could then act as new foci for outgrowth, and restriction of extension

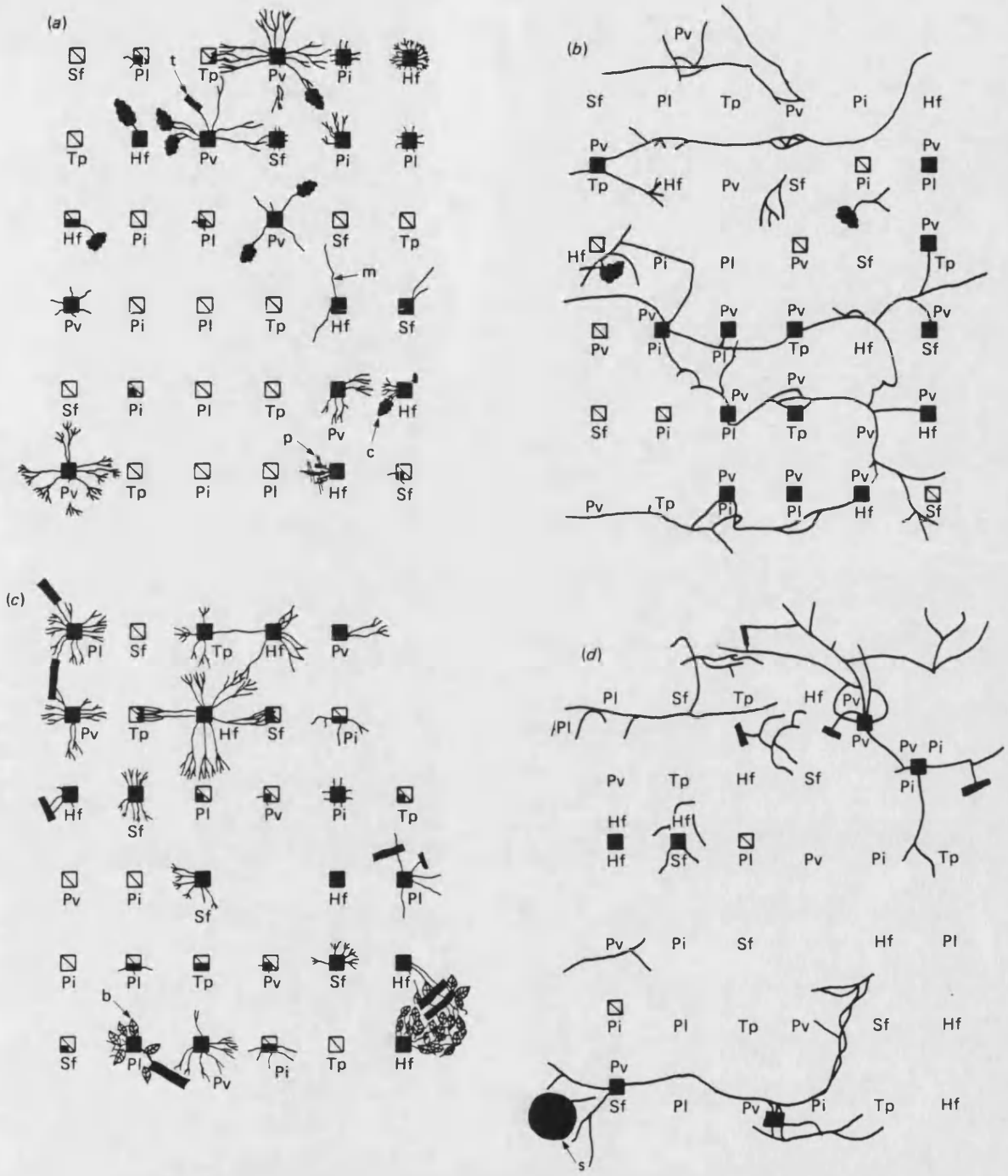

Figure 2. Development of mycelial systems of *Hypholoma fasciculare* (Hf), *Phallus impudicus* (Pi), *Phanerochaete laevis* (Pl), *Ph. velutina* (Pv) and *Steccherinum fimbriatum* (Sf) in multiple arrays (arrangement 2). (*a*) 6 months and (*b*) 2 years after inoculation at site A during December. (*c*) 3 months and (*d*) 2 years from inoculation at site B during March. Inoculum block colonized by a cord-former (■, subscript original cord, superscript replacing cord). Block surface 50% colonized by a cord-former (◪) and 25% colonized (◪). Block colonized by a non-cord-former (▨). Block decayed, with subscript block missing, without subscript; Mycelial cord (m), *Pinus cone* (c), twig (t), *Fagus* leaves (b), *Pinus* needles (p), Stump (s). Blocks 14 × 17 cm apart.

along a row by a neighbour, so redirecting growth to another row. This is illustrated in Figure 2(*c*, *d*) which shows outgrowth of *H. fasciculare* at site B clearly restricting the growth of *Ph. velutina* in several areas, but not at site A where *H. fasciculare* did not establish [Fig. 2(*a*,*b*)]. Both arrays were inoculated in winter and there was little if any extension of the other strains. However, following spring inoculation, *Ph. laevis* and *S. fimbriatum* both showed improved extension (see Dowson *et al.*, 1988*a*) and restricted colonization and replacement by *Ph. velutina*, although after 2 years, at site A, B

and E, only systems of *S. fimbriatum* were found in close proximity to *Ph. velutina.*

CONCLUSIONS

The results of the experiments described in this and the previous paper (Dowson *et al.*, 1988*a*) demonstrate the influence of three interconnected primary determinants of the establishment and persistence of mycelial cord systems on the woodland floor: microenvironment, resource availability and incidence of competitors. This accords with the concept of three primary ecological strategies, ruderal (R), stress-tolerant (S) and combative (C), conditioned by the relative occurrence of environmental stress and disturbance (see Grime, 1979; Pugh, 1980; Cooke & Rayner, 1984; Coates & Rayner, 1985). Each of the species examined may be considered to exhibit a different spectrum of R, C and S characteristics, and phases of establishment, resource capture and persistence may be affected differentially by environmental determinants.

Initial outgrowth was affected primarily by micro-environmental conditions, as indicated by the differences between winter and spring inoculations and between sites. The intrinsic capacity for early outgrowth on a broad mycelial front may be linked to the predictability of locating suitable resources for colonization in proximity to the inoculum bases (Rayner & Franks, 1987). Such predictability is enhanced by possession of 'unspecialised' resource relationships (Rayner, Watling & Frankland, 1985) and the combative ability to replace resident organisms. The extensive outgrowth of *Ph. velutina*, *H. fasciculare* and *S. fimbriatum* can be understood in these terms, although that of the latter species depends also on particular micro-environmental conditions (fluctuating soil moisture). On the other hand, the 'poor' performance of *Phallus. impudicus* and *T. platyphylla* may be linked with more 'specialised' resource relationships and dependence on the absence of competitors.

The importance of combative ability in resource capture and persistence was indicated by the behaviour of *Ph. velutina* which often formed extensive systems in arrangement 2 experiments at the expense of the other species, including *H. fasciculare* which is usually an effective combatant against other fungi. On certain sites, however, *S. fimbriatum* formed extensive systems in competition with *Ph. velutina*, and this was linked to its strong defensive capability in retaining colonized resources.

An experimental analysis of the mechanisms and outcomes of combative interactions involving mycelial-cord forming fungi during growth through soil and on artifical media is the subject of a subsequent paper (Dowson, Rayner & Boddy, 1988*b*).

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CHAPTER 4.

New Phytol. (1988), **109**, 423–432

The form and outcome of mycelial interactions involving cord-forming decomposer basidiomycetes in homogeneous and heterogeneous environments

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SUMMARY

The mycelial interactions of strains of six cord-forming wood-inhabiting basidiomycetes were studied both against each other and against other fungi including *Armillaria* species on 2% malt extract agar, in wood lengths, and in non-sterile soil. Generally, cord-formers could be ranked in a combative order *Phanerochaete velutina* (DC ex Pers.) Parmasto = *Phanerochaete laevis* (Fr.) Eriksss. & Ryv. > *Steccherinum fimbriatum* (Pers. ex Fr.) Eriksss. = *Hypholoma fasciculare* (Huds. ex Fr.) Kummer = *Phallus impudicus* (L.) Pers. > *Tricholomopsis platyphylla* (Pers. ex Fr.) Sing. However, the outcome of interactions varied considerably according to circumstances. For example, in soil systems it depended on the extent to which encounters occurred between mycelia growing out from the wood inoculum blocks or within the inocula themselves. This depended in turn on the relative size of the inoculum blocks used for each strain.

Encounters between like mycelia growing out into soil led to the formation of persistent mycelial connectives between the inoculum blocks. However, those between unlike mycelia elicited discolouration and lytic reactions following either contact ('mycelial interference') between different species, or fusion and somatic incompatibility between different strains of the same species. Such reactions were followed either by replacement of one system by the other, or the development of mycelium-free zones of soil between deadlocked colonies.

After 3 months all pairings of cord formers against systems of *Armillaria bulbosa* (Barla) Kile and Watling, *A. cepestipes* Vel. f. *pseudobulbosa* Romagn. & Marxmuller and *A. mellea* (Vahl ex Fr.) Kummer in soil resulted in the colonisation of the *Armillaria* inocula to varying degrees and the death of virtually all associated rhizomorphs.

Key words: *Armillaria*, biological control, fungal interactions, mycelial cords, soil fungi.

INTRODUCTION

Dowson, Rayner & Boddy (1988*a*, *b*) considered the direct inoculation of cord-forming basidiomycetes into woodland soil as a possible means of biological control of forest pathogens and/or manipulating nutrient cycling. Their results demonstrated a crucial role for interactions of these fungi, both with each other and with other soil and litter inhabitants, in determining patterns of persistence and mobility on the woodland floor.

In this paper we describe a laboratory-based analysis of the mechanisms and outcomes of interactions involving cord-forming basidiomycetes and how these are affected by the circumstances under which growth occurs. In particular, we have tried to simulate the spatial discontinuity in resource supply and environmental heterogeneity which characterise natural habitats.

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MATERIALS AND METHODS

Fungal strains

Table 1 lists the fungal strains together with an indication of their origin and the experiments for which they were used. All strains were routinely cultured on 2% malt extract agar (MEA, 20 g

Table 1. *Fungal strains used in interaction experiments*

Ecological category	Fungus	No. of strains	Experiments*	Isolated from†	Sites (N.G. ref.)
Rhizomorph-forming wood-decomposers/ tree pathogens	*Armillaria bulbosa* (Barla) Kile & Watling	1	S	M	SO 611145
	Armillaria cepestipes Vel. f. pseudobulbosa Romagn. & Marxmuller	1	S	M	NS 356075
	Armillaria mellea (Vahl ex Fr.) Kummer	1	S	F	'Cambridge'
Woodland-litter decomposers	*Clitocybe flaccida*(Sow. ex Fr.) Kummer	1	MEA	F	SU 008631
	Clitocybe nebularis (Batsch. ex Fr.) Kummer	1	MEA	F	ST 785590
	Collybia butyracea (Bull. ex Fr.) Kummer	1	MEA	F	ST 787590
	Collybia dryophila (Bull. ex Fr.) Kummer	1	MEA	F	ST 787588
	Collybia maculata (Alb. & Schw. ex. Fr.) Kummer	1	MEA	F	Suffolk
	Coprinus picaceus (Bull. ex Fr.) S. F. Gray	1	MEA	W	ST 787588
	Mycena galopus (Pers. ex Fr.) Kummer	1	MEA	W	ST 785588
Grassland-litter decomposer	*Coprinus comatus*(Mull. ex Fr.) S. F. Gray	1	MEA	F	ST 784585
Non-cord-forming wood decomposers	*Psathyrella candolleana*(Fr.) Maire	1	MEA	F	ST 785590
	Tricholomopsis rutilans(Pers. ex Fr.) Sing.	1	MEA	F	ST 785591
Cord-forming wood decomposers	*Cristella farinacea*(Pers. ex Fr.) Donk	1	MEA	W	ST 798725
	Cristella sulphurea (Pers. ex Fr.) Donk	1	MEA	M	ST 784593
	Hypholoma fasciculare (Huds. ex Fr.) Kummer	3	MEA, B, S	W	SO 611145 ST 784593 ST 798725
	Phallus impudicus (L.) Pers.	3	MEA, B, S	M	SU 008631 ST 785592 ST 785590
	Phanerochaete laevis (Fr.) Erikss.	1	MEA, B, S	W	ST 795563
	Phanerochaete velutina (DC ex Pers.) Parmasto	3	MEA, B, S	W	ST 795563
	Steccherinum fimbriatum (Pers. ex. Fr.) Erikss.	1	MEA, B, S	W	ST 795563
	Tricholomopsis platyphylla (Pers. ex Fr.) Sing.	1	MEA, B, S	W	SU 213682

* MEA, pairings on malt extract agar; B, pairings in beech wood lengths; S, pairings in soil. Where more than one strain is listed, these were used in intraspecific pairings in soil.

† M, mycelium; F, fruit body, W, decaying wood.

Munton & Fison spray malt A, 15 g Lab-M agar No. 2 per litre distilled water).

Experimental pairings

Three types of experiments were performed, all in triplicate, as follows.

On MEA. Mycelial discs were cut from the actively growing margin of fungal colonies and placed, 3 cm apart, on 9 cm Petri plates. Slow-growing strains were inoculated prior to faster-growing ones, so that the mycelia met initially at the centre of the plates. The plates were incubated in darkness for up to 11 weeks at 15 °C.

In beech lengths. Straight branches, 1·5 cm in diameter, were selected from a freshly felled beech tree (*Fagus sylvatica* L.), and cut into lengths of 7 cm. Holes, 1 cm in diameter and 1·5 cm deep, were drilled into the ends of each length. The lengths were then autoclaved in sealed plastic bags for three periods of 30 min at 121 °C. Colonized wood dowels of rhamin (*Gonystylus macrophyllum*), obtained by incubation for 2 weeks at 20 °C in darkness on the relevant mycelia in 9 cm Petri plates, were placed into the holes at the end of each beech length, at times calculated to result in an interaction interface about mid-way down the length. The inoculated lengths were then incubated for up to 4 months at 15 °C in

darkness in sterile covered plastic trays (24 × 24 cm) enclosed in polythene bags to maintain humidity. The lengths were sectioned longitudinally and, before re-assembling and re-incubating them, fragments excised from the bark or wood were plated onto MEA. The mycelia recovered were identified by pairing against the original strain and noting whether or not an intermingling reaction resulted.

In soil. Eight cm^3 beech inoculum wood blocks colonized by the cord-formers and *Armillaria* species were prepared as described by Dowson *et al.* (1988*a*). Pairings were made by placing different combinations of inoculum blocks on opposite sides of 14 cm diameter Petri dishes filled with a sieved sandy loam soil, initially at 37 % moisture (field capacity). Alternatively the blocks were cut into 1 cm^3 portions and these portions placed singly, or in groups of eight, in soil-filled dishes as described above. Inoculations were generally timed to allow meeting of mycelia in the centre of the plates. *Armillaria* spp. were inoculated 2 months prior to the cord formers to allow a substantial network of rhizomorphs to develop.

The plates were incubated in darkness at 15 °C for up to 6 months, during which time they were periodically weighed and re-wetted with a fine spray to maintain soil moisture between 30 and 37 % (oven-dry weight). Mycelial progress across the soil surface and the underside of the plates was recorded every 4–6 weeks. At the end of the experiment the inoculum blocks were sectioned and fungal cultures re-isolated from them onto MEA. The cultures were identified by pairing with the original strains.

RESULTS

Interactions on 2% malt agar

Self-pairings intermingled to produce uniform mycelial mats. Non-self interactions on MEA could generally be classified either into 'deadlock' reactions, where neither mycelium visibly entered the domain occupied by the other, or 'replacement' where one mycelium encroached into the other colony, hence partially or totally taking over its domain (Table 2). Subcultures from replacement fronts yielded only the dominant strain. However, the mechanisms underlying the gross outcome of interactions varied (Fig. 1). Occasionally, i.e. between *Phallus impudicus* and *Clitocybe flaccida* (Fig. 1*a*) and between *Tricholomopsis platyphylla* and *Collybia maculata*, mutual inhibition of radial extension prior to contact resulted in deadlock. However, although some marginal inhibition of *Hypholoma fasciculare* occurred when it was paired against *Clitocybe flaccida*, the remaining interactions only developed following contact between mycelia. These often involved the formation of dense mycelial fronts at the interface which either resisted mycelial penetration, or were responsible themselves for overgrowth or throughgrowth of opposing colonies. In some cases these fronts were not aggregated into discrete linear structures, as in the interactions between *Steccherinum fimbriatum* and *Tricholomopsis platyphylla* (Fig. 1*b*), *T. platyphylla* and *Psathyrella candolleana*, and *Phallus impudicus* and *Cristella farinacea* (Fig. 1*c*). In the latter interaction, the normally cord-like growth of *C. farinacea* locally switched to a more uniform mycelial front which

Table 2. *Outcome of interactions on 2% malt agar related to cord formers**

	Cord formers					
	H. fasciculare	*P. impudicus*	*P. laevis*	*P. velutina*	*S. fimbriatum*	*T. platyphylla*
Hypholoma fasciculare	i	r	r	R	r	r
Phallus impudicus	R	i	R	R	R	d
Phanerochaete laevis	R	r	i	r	r	—
Phanerochaete velutina	r	r	R	i	r	r
Steccherinum fimbriatum	R	r	R	R	i	d
Tricholomopsis platyphylla	R	d	—	R	d	i
Cristella farinacea	R	R	—	R	R	R
Cristella sulphurea	R	—	d	R	R	r
Clitocybe flaccida	R	d	R	R	R	R
Clitocybe nebularis	R	R	R	R	R	R
Collybia butyracea	R	d	—	R	R	R
Collybia dryophila	R	d	R	R	R	—
Collybia maculata	R	R	—	R	R	R
Coprinus comatus	R	—	R	R	r	r
Coprinus picaceus	r	r	r	d	r	r
Mycena galopus	d	r	R	R	d	r
Psathyrella candolleana	R	R	—	R	R	R
Tricholomopsis rutilans	—	r	R	R	r	—

* Results relating to the cord formers: R, replacing; r, being replaced; d, deadlock; i, intermingling; —, data absent.

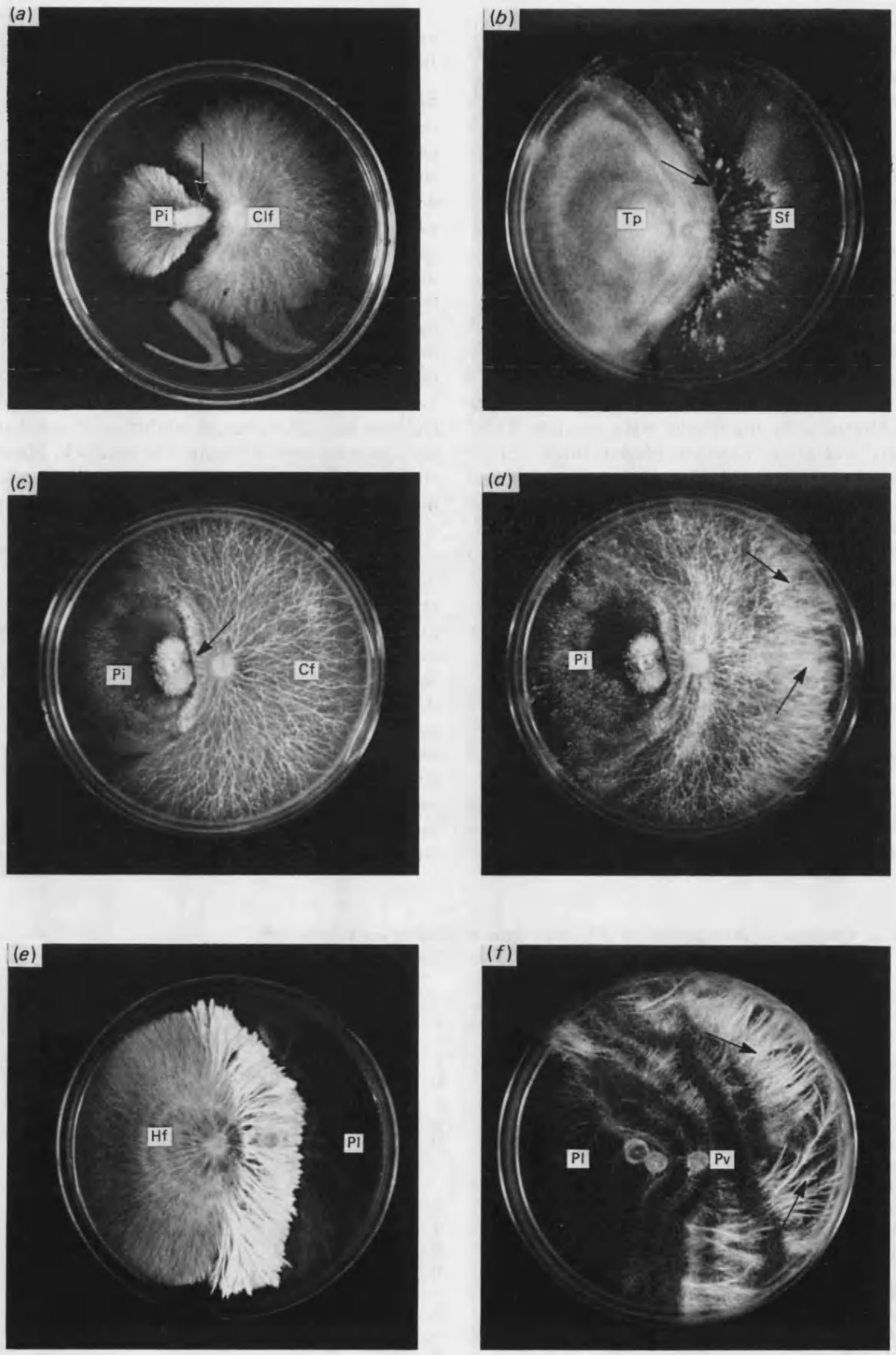

Figure 1. Interactions of cord-forming fungi on 2% malt agar. (*a*) Pre-contact inhibition (arrowed) between *Phallus impudicus* (Pi) and *Clitocybe flaccida* (Clf). (*b*) Post-contact replacement (arrowed) of *Steccherinum fimbriatum* (Sf) by *Tricholomopsis platyphylla* (Tp). (*c*) Initial overgrowth (arrowed) of *Phallus impudicus* (Pi) by *Cristella farinacea* (Cf) after 4 weeks. (*d*) Eventual replacement of *C. farinacea* by *P. impudicus* (Pi) with throughgrowth of cords (arrowed) after 10 weeks. (*e, f*) Cord-mediated replacement of (*e*) *Phanerochaete laevis* (Pl) by *Hypholoma fasciculare* (Hf), (f) *Phanerochaete velutina* (Pv) by throughgrowth (arrowed) of *P. laevis* (Pl).

Table 3. *Outcome of interactions between cord-forming fungi in beech wood lengths*

	**S. fimbriatum*	*P. velutina*	*P. laevis*	*P. impudicus*	*H. fasciculare*
Hypholoma fasciculare	R	R	R	r	i
Phallus impudicus	R	R	R	i	R
Phanerochaete laevis	R	r	i	r	r
Phanerochaete velutina	R	i	R	r	r
Steccherinum fimbriatum	i	r	r	r	r

* Strains listed at the head of the table: R, replacing; r, being replaced; i, intermingling.

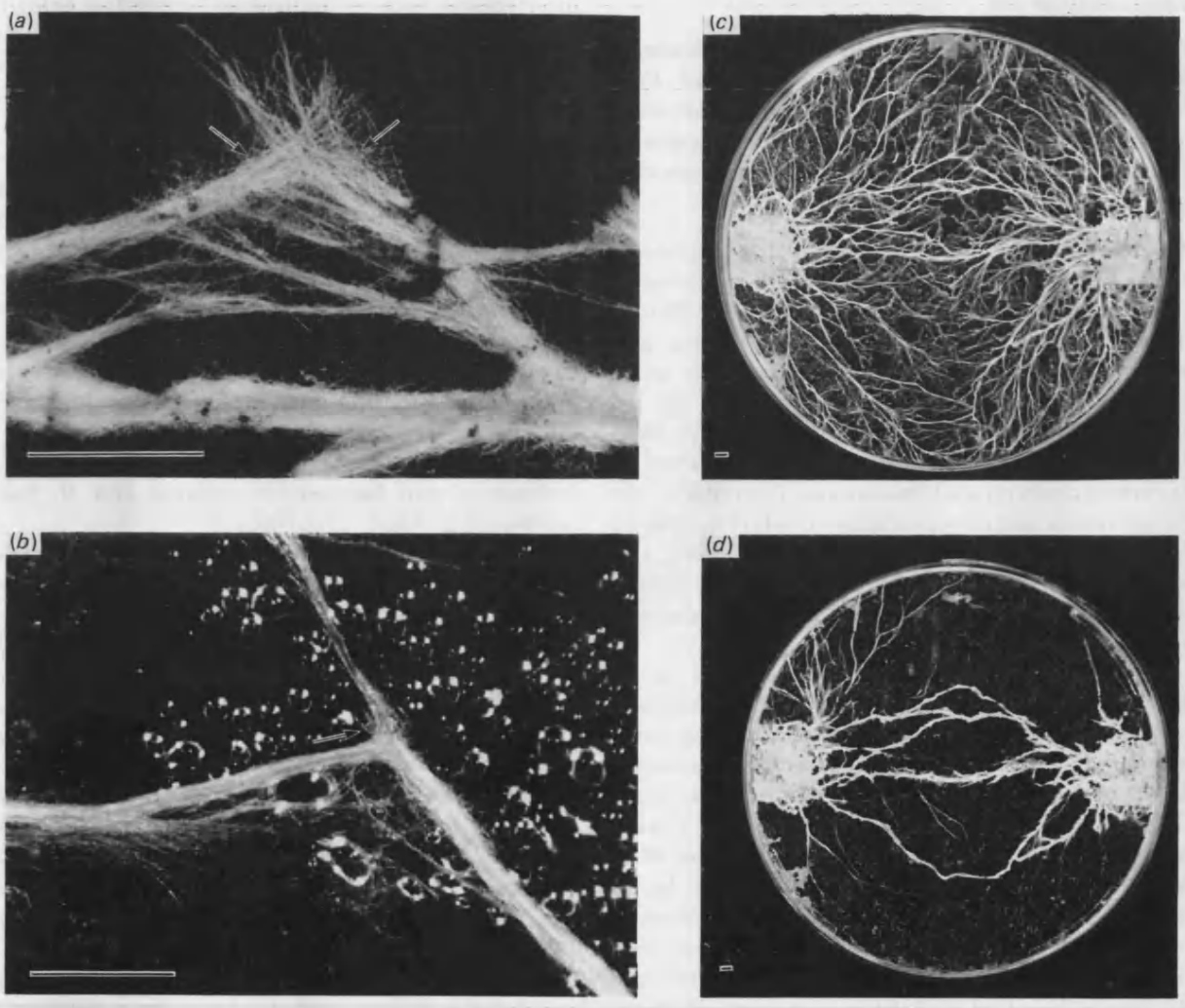

Figure 2. Formation of interconnections between genetically identical mycelia of cord-forming fungi grown in 14 cm diameter soil trays at 15 °C. (*a*) Tip-to-tip fusion in *Phanerochaete velutina* (arrowed). (*b*) Tip-to-side fusion in *Steccherinum fimbriatum*. (*c*) Interconnecting cords of *P. velutina* after 3 weeks and (*d*) after 12 weeks, showing areas where lysis has occurred and connecting cords have thickened. Scale bar represents 2 mm.

encroached over the initially uniform mat of *P. impudicus*. Subsequently, however, *P. impudicus* produced a mycelial front consisting of cords which fully penetrated the *C. farinacea* colony (Fig. 1*d*). This subsequent behaviour of *P. impudicus* reflected the more general responses to interaction of cord-forming fungi (except *T. platyphylla*), that is the production of invasion fronts consisting of discrete linear aggregations [further examples are shown in Fig. 1(*e*) and (*f*)].

Interactions in beech lengths

By contrast with interactions on MEA and in soil (see below), confrontations between cord-forming fungi in beech lengths always appeared to result in

complete replacement rather than deadlock. When the lengths were sectioned longitudinally, no interaction zones demarcating different mycelia were evident before or after re-incubation and only one strain was recovered by subculturing from each non-self pairing, whereas both strains were readily recovered from control self-pairings. The results are shown in Table 3, from which it can be deduced that *Steccherinum fimbriatum* most consistently replaced other strains.

Interactions in soil

General features. Hypholoma fasciculare, Phallus impudicus, Phanerochaete laevis, P. velutina and *Steccherinum fimbriatum* all readily produced cords when inoculated on wood blocks into soil, and opposing systems normally made contact with one another after 1 or 2 months.

Intraspecific interactions. In self-pairings, fusions occurred between cords which met and intermeshed either tip-to-tip (Fig. 2*a*) or tip-to-side (Fig. 2*b*). As time proceeded the fused hyphal aggregates persisted, while peripheral hyphae present on initial contact (Fig. 2*a*) disappeared, resulting in the establishment of extensive networks of cords (Fig. 2*c*). Further development resulted in the thickening of certain connectives between inoculum blocks and lysis of minor and non-connective cords (Fig. 2*d*). In both *S. fimbriatum* and *Phanerochaete laevis* this process of lysis occurred until eventually only a single interconnecting cord persisted, either above or below the soil surface respectively.

By contrast, non-self pairings, observed in *H. fasciculare, Phallus impudicus* and *Phanerochaete velutina*, invariably resulted in deadlock interactions, with cords of both strains undergoing substantial discolouration and lysis at the confrontation zone. Consequently, zones of mycelium-free soil, 1–4 cm wide and noticeably darkly stained in the case of *P. velutina*, developed between the colonies (Fig. 3*a, b*). By contrast with interspecific deadlock interactions (see below), continued extension into the confrontation zones was infrequent, and prolonged incubation resulted in further regression of initial outgrowth.

Interspecific interactions between cord-forming fungi. As on agar, mycelial interactions in soil produced both deadlock and replacement outcomes (Table 4). However, these outcomes were mediated in two distinct stages, firstly between the mycelial outgrowth systems and secondarily within the inoculum blocks.

Contact between outgrowth systems characteristically resulted in marked yellow or brown discolouration and lytic responses in one or both of the mycelial cord segments involved (Fig. 3*c–h*). Such responses, which may be termed 'mycelial interference' to indicate their affinity with 'hyphal interference' between individual hyphae (see below), were often clearly visible up to 1 cm from the point of contact. Segments of cords distal to these responses usually died unless linked by anastomoses to neighbouring unaffected cords.

Deadlock sometimes resulted when mutual mycelial interference was sufficient to prevent both strains from penetrating beyond the confrontation zone and reaching the opposing inoculum block. Examples were provided by *H. fasciculare* versus *P. impudicus*, *H. fasciculare* versus *S. fimbriatum* below the soil surface (Fig. 3*c, d*) and *P. laevis* versus *S. fimbriatum*. In other cases, cords of one of both strains were able to reach the opposing inoculum block but unable to colonize it because of mycelial interference reactions, e.g. between *P. impudicus* and *H. fasciculare* or *S. fimbriatum*, and between *P. velutina* and *S. fimbriatum* (Fig. 3*e*).

Replacement sometimes occurred despite apparently mutual interference between mycelial outgrowth systems. In the interactions between *P. laevis* and *S. fimbriatum* deadlock at the mycelial interface occasionally broke down, so that one or more cords of either species reached the opposing inoculum block. *P. laevis* was usually unable to colonise inoculum blocks of *S. fimbriatum*, but *S. fimbriatum* was able to replace *P. laevis* once contact with the inoculum block occurred. In pairings between *S. fimbriatum* and *H. fasciculare* where deadlock occurred below the soil surface (see above, Fig. 3*c, d*), replacement of *H. fasciculare* in its inoculum block occurred following extension of *S. fimbriatum* cords above the soil surface where they avoided interference with *H. fasciculare* cords.

Other replacement reactions followed unilateral mycelial interference responses between cord systems, e.g. between *P. velutina* and *P. impudicus* (Fig. 3*f, g*) and between *P. laevis* and *P. velutina* (Fig. 3*h*).

Figure 3. (*a, b*) Intraspecific interactions between genetically non-identical heterokaryotic strains of *Phanerochaete velutina* growing from wood block inocula in soil incubated at 15 °C. (*a*) Initial contact resulting in a zone of interference with staining and lysis. (*b*) The same interaction 5 weeks later showing development a mycelium-free zone between the two strains. (*c–h*). Interspecific interactions of cord-forming fungi in soil. (*c*) Deadlock between slow dense mycelia of *Hypholoma fasciculare* (Hf) and cords of *Steccherinum fimbriatum* (Sf) below the soil surface (arrowed). (*d*) Staining in *H. fasciculare* (arrowed) in the same interaction. (*e*) Interference reactions between cords of *P. velutina* (Pv) and S. *fimbriatum* (Sf), showing yellowing of *S. fimbriatum* (arrowed). (*f, g*) Replacement of *P. impudicus* by *P. velutina* (Pv). (*f*) Initial contact and staining of *P. impudicus* (arrowed). (*g*) Lysis of cords of *P. impudicus* and either interference or replacement at the inoculum block. (*h*) Differentiation in tips of *P. laevis* (Pl) when replacing *P. velutina*, showing fine red hyphae (arrowed) growing over *P. velutina*. Scale bar represents 2 mm.

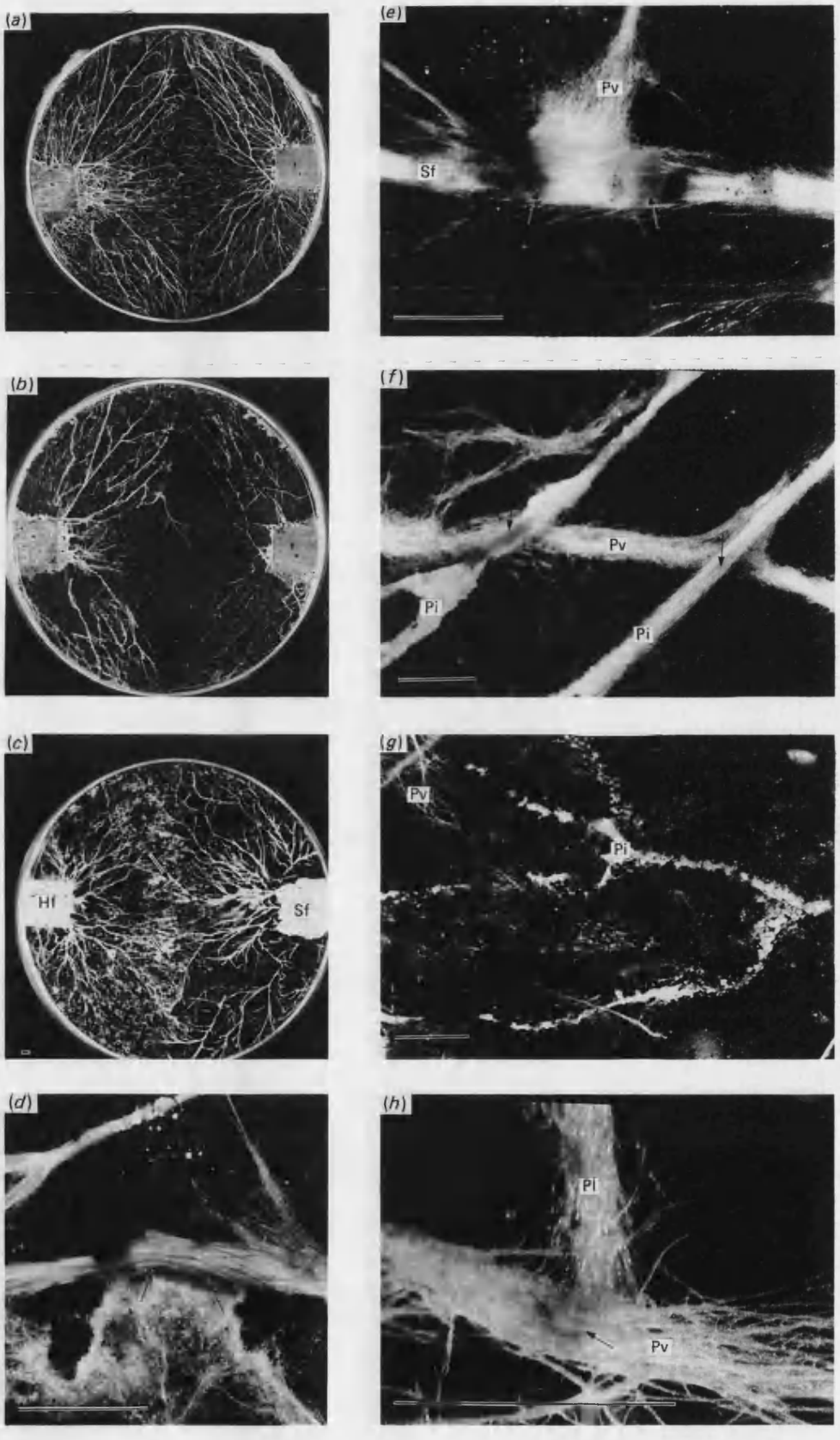
(a)
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Table 4. *Outcome of interspecific interactions between cord-forming fungi in soil*

	T. platyphylla* in blocks	S. fimbriatum		P. velutina		P. laevis		P. impudicus		H. fasciculare	
		Between cords	In blocks	Between cords	In blocks	Between cords	In blocks	Between cords	In blocks	Between cords	In blocks
Hypholoma fasciculare	r	d	R	R	R	R	R	r	d	i	i
Phallus impudicus	r	r	d	R	R	R or d	R	i	i	R	d
Phanerochaete laevis	r	r or d	R	r	r (R)	i	i	r	r	r	r (d)
Phanerochaete velutina	r	d	d	i	i	R	R	r	r	r	r (d)
Steccherinum fimbriatum	r	i	i	d	d (R)	R or d	r (R)	R	d	d	r (R)
Tricholomopsis platyphylla	r	—	R	—	R	—	R	—	R	—	R

* Strains listed at head of table were replacing (R), being replaced (r), deadlocking (d) or intermingling (i), —, data absent. Letters in parentheses indicate a change in outcome when inocula of strains at the head of the table were eight times larger than the other, rather than the same size. Absence of parentheses indicates no change in outcome.

Table 5. *Outcome of interactions between cord-forming fungi and* Armillaria *species in soil*

	Armillaria bulbosa			Armillaria cepestipes			Armillaria mellea		
	Block covered (%)*	Depth penetrated (mm)	No. viable rhizo-morphs†	Block covered (%)*	Depth penetrated (mm)	No. viable rhizo-morphs†	Block covered (%)*	Depth penetrated (mm)*	No. viable rhizo-morphs†
Hypholoma fasciculare	100	2	0	100	2	0	100	2	0
Phallus impudicus	50	< 1	0	80	< 1	0	—	—	—
Phanerochaete laevis	70	4	0	100	5	0	100	5	0
Phanerochaete velutina	100	2	0	100	2	< 1	100	2	0
Steccherinum fimbriatum	50	< 1	< 1	100	< 1	3	5	< 1	—

* Mean values from triplicates. The data relate to colonization by cord formers.
† Controls without cord formers possessed numerous viable rhizomorphs.

In the latter case, *P. laevis* produced narrow red hyphae which overgrew the opposing cord; in other confrontations *P. laevis* responded to contact by producing bushy salmon-pink mycelial apices.

In all replacement reactions, once cords of the dominant species made contact with the opposing inoculum block, colonization was rapid and resulted in total lysis of the associated cord system (Fig. 3*g*). Replacement culminated in the emergence of the dominant species as a fan of mycelial cords from the colonized block. Replacement of other species by *S. fimbriatum and P. laevis* was generally brought about following arrival at the inoculum block of a single cord, whilst replacement by *P. velutina* usually followed arrival by several cords.

The outcome of an interaction, from one of being replaced to deadlock or even to replacement, could often be altered by increasing the size of the inoculum base of one species relative to the other. For example *H. fasciculare* replaced *S. fimbriatum* when the ratio between the respective inoculum sizes was 8:1, but *S. fimbriatum* replaced *H. fasciculare* when the ratio was 1:1. The combative abilities of *P. laevis*, *P. velutina* and *S. fimbriatum* were similarly improved by increasing their relative inoculum size, but not that of *P. impudicus*.

Interactions with Armillaria *species.* In all pairings of cord-formers (except *T. platyphylla*) against *A. bulbosa*, *A. cepestipes* and *A. mellea*, inoculum blocks of the *Armillaria* species were colonized to varying degrees by the cord-former after 3 months and most rhizomorphs had lost viability, becoming blackened and fragile (Table 5). In control experiments, where *Armillaria* was inoculated in the absence of cord-formers, numerous, vigorous rhizomorphs were present after this time.

H. fasciculare fully colonized the block surface of all the *Armillaria* species to a depth of 2–3 mm, and no viable rhizomorphs remained. The zone of colonization was clearly delimited within the blocks by a single black pseudosclerotial plate produced by residual viable *Armillaria* mycelium.

P. velutina and *P. laevis* showed patterns of colonization similar to *H. fasciculare*, but one or two viable rhizomorphs of *A. bulbosa* and *A. cepestipes* remained in pairings against *P. velutina*, and penetration of *A. mellea*–inoculated blocks by *P. laevis* occurred to a depth of up to 5 mm.

Colonization of inoculum blocks of *A. bulbosa* and *A. cepestipes* by *P. impudicus* was incomplete, 30 and 75 % of the surface area respectively being covered, and penetration only occurred to a depth of 1–2 mm. Even so, only a single viable rhizomorph of *A. cepestipes* remained.

S. fimbriatum was the weakest combatant against *Armillaria*, covering only 25 % of each block of *A. mellea*, 25–75 % of *A. bulbosa* and 80–100 % of *A. cepestipes*, with several viable rhizomorphs remaining in dishes containing *A. cepestipes* and *A. mellea*.

DISCUSSION

The range of outcomes of interactions and mechanisms observed during the present study corresponds with those reviewed by, for example, Rayner & Webber (1984) and Rayner (1986). Overall, the cord-forming species could be ranked in a combative order as follows: *Phanerochaete velutina* = *Phanerochaete laevis* > *Steccherinum fimbriatum* = *Hypholoma fasciculare* = *Phallus impudicus* > *Tricholomopsis platyphylla*. Moreover, most were strongly to moderately combative against non-cord-forming fungi and *Armillaria* species, as would befit their free-ranging life style on the forest floor. The differences amongst the cord-forming species themselves correspond with observations of their patterns of persistence and resource capture over a prolonged period (Dowson *et al.*, 1988*b*), and may be related to differences in ecological strategy. Broadly, those with the least specialized resource relationships appear to possess the most aggressive combative strategies, whilst those with more specialized resource relations possess lesser or more defensive combative abilities. There may also be some correlation with the form of mycelial outgrowth, the more highly rhizomorphic (more apically dominant) foraging patterns being more specialized than those spreading as fans on a broad front (Rayner & Franks, 1987; Dowson, Rayner & Boddy, 1988*c*).

Although there was a broad correspondence between outcome of interactions observed on agar, in wood and in soil, the mode of development of the interactions often differed substantially. Moreover, the replacement of other species in wood by *S. fimbriatum* contrasted strongly with its performance on agar and in soil. These differences are probably partly a reflection of the varying developmental status of mycelia at the interaction interface. On agar initial encounters were generally between diffuse mycelia, but final outcomes often reflected changes in one or both participants to more compacted patterns of morphogenesis.

The reverse situation applied in soil, where initial encounters were between hyphal aggregates supplied with resources from the inoculum wood blocks. Hence it was of interest to observe interactions between the aggregated systems which may be considered analogous to, or an amplification of, responses which are well known to occur between individual hyphae. Thus the somatic incompatibility response which is confined to the non-self fusion compartments, or at most a few neighbouring compartments in hyphae of *Phanerochaete velutina* (Ainsworth & Rayner, 1986), extended along fused mycelial cord segments as long as 4 cm in the soil dishes (see also Thompson & Rayner, 1983). Simi-

larly the 'mycelial interference' reactions between different species closely paralleled the hyphal interference reaction where hyphal contact is followed by death of the protoplasm in one or both of the hyphal compartments involved (Ikediugwu & Webster, 1970*a*, *b*; Traquair & McKeen, 1977).

It may be assumed that mycelial encounters within wood lengths are primarily between diffuse systems, although these may later be reinforced by formation of pseudosclerotial plates. *S. fimbriatum* appears to be at a distinct advantage in this situation, as is evident from its replacement of all other species in beech lengths, and of other species in inoculum blocks in soil, if it successfully bypassed their mycelial outgrowth systems.

These relationships between mycelial development and interactions reinforce the need to think of fungal mycelia in their natural habitats as versatile collectives of semi-autonomous units (hyphae) able to bridge heterogeneous and spatially discontinuous sites of activity. As between two armies, interactions may occur both at the level of skirmishes between individual troops (hyphae) or co-ordinated sections which may in turn be functionally specialized (differentiated) for quite differing activities.

One of the more practical implications of this study concerns the possible use of cord-forming fungi for biological control of *Armillaria* root pathogens. Evidently the cord formers are capable at least of partial replacement of *Armillaria* in wood, until arrested by pseudosclerotial plates, and certainly of immobilizing *Armillaria* through prevention or destruction of outgrowth of rhizomorphs. However, their ability to restrict outgrowth of rhizomorphs from submerged roots may be limited by microenvironmental conditions. Such wood, when decayed by *Armillaria*, is usually water-saturated and may contain significant amounts of antibiotics (Oduro, Munnecke, Sims & Keen, 1976). Studies by Chapela, Boddy & Rayner (1988) have indicated that the combative ability of cord-formers on agar is reduced significantly by high CO_2/low O_2 concentrations, and this may restrict containment or replacement of *Armillaria* in submerged roots.

In a study of the colonization of light-suppressed trees in established woodland, Thompson & Boddy (1983) found that the root collar and shallow roots were often extensively colonized by cord-formers, but deeper roots were occupied virtually exclusively by *Armillaria bulbosa* or *Armillaria ostoyae* (Romagn.) Herink. Such roots may still function as infection foci since although rhizomorphs usually occur in the upper soil horizons (2·5–20 cm deep) of both heavy clay and light soils (Rishbeth, 1982) they have also been excavated from depths of up to 60 cm (Ono, 1965; Redfern, 1973).

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CHAPTER 5.

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Outgrowth Patterns of Mycelial Cord-forming Basidiomycetes from and between Woody Resource Units in Soil

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Wood blocks colonized by the basidiomycetes *Hypholoma fasciculare* and *Phanerochaete velutina* were placed in plastic trays containing moist unsterilized soil. Both fungi grew out radially from the inoculum blocks in the form of networks of mycelial cords. When a second, uncolonized wood block, or set of wood blocks, was provided as a 'bait' about 5 cm from the inoculum block, marked changes in the form and growth characteristics of the mycelial network followed contact with the bait. These changes were influenced by the relative size of inoculum and bait and included inhibition of radial extension from the inoculum; stimulation of development of connective mycelium; directed growth responses to the bait; fan-shaped outgrowth with conserved polarity from the bait; eventual regression of non-connective mycelium originating from the inoculum. These effects presumably reflect the capacity of the mycelium to behave as a co-ordinated unit and to economize on biomass when growing between discontinuously supplied resource units.

INTRODUCTION

Physiological studies of the growth of mycelial fungi have largely been made using artificial media in which nutrients are supplied homogeneously. These studies have given rise to the concept that fungal mycelia can be envisaged primarily as collections of independently growing and duplicating units ('hyphal growth units'), each consisting of a hyphal tip together with an unspecified length of hypha (Trinci, 1978, 1979). Although this concept may well be appropriate for many fungi growing under at least initially homogeneous conditions, in nature it is common for nutrient resources to be discontinuously supplied, both in space, as separate resource units, and in time (Cooke & Rayner, 1984). Under these circumstances there will be pressure on the mycelium for economical discovery, exploitation and allocation of available nutrients, and this might be expected to have a considerable impact on growth patterns and co-ordination of activity (Dowding, 1976, 1981; Watkinson, 1984; Thompson, 1984).

Few attempts have been made to study growth patterns between discontinuously supplied resource units experimentally. One group of fungi which habitually grow in this way are certain wood and litter-rotting species, especially of Basidiomycotina, which form mycelial cords. The latter consist of aggregations of predominantly parallel, longitudinally aligned hyphae, and are often differentiated into a distinct outer crust with a high proportion of wide vessel hyphae within its core (Thompson & Rayner, 1982*a*, 1983). They form extensive mycelial systems interconnecting pieces of decaying substratum on the woodland floor (Thompson & Rayner, 1982*a*, *b*, 1983; Thompson, 1984), and their large size and ability to grow directly into non-sterile soil from wood block inocula makes them ideal for experimental studies. Here we report one such study, using *Hypholoma fasciculare* and *Phanerochaete velutina*, which are both widespread agents of decay of wood of deciduous trees in contact with the woodland floor in Britain.

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METHODS

Strains and culture media. Hypholoma fasciculare was isolated from tissue of a fruit body produced on a beech (*Fagus sylvatica*) log collected from Sallowvallets Inclosure, Forest of Dean, Glos., UK (National Grid reference SO 611145). *Phanerochaete velutina* was isolated from decayed wood of *F. sylvatica* collected from Farleigh Hungerford Woods, Wilts., UK (ST 795563). Both fungi were routinely cultured on 2% (w/v) malt extract agar (MA; 20 g Munton & Fison spray malt A and 15 g Lab-M agar no. 2 per litre).

Preparation of wood block inocula and baits. Wood blocks approximately 8 cm^3 were cut from freshly felled trees (approximately 15 cm diameter) of *F. sylvatica* from Colerne Woods, near Bath (ST 798725). The blocks were stored at −18 °C. Before use they were thawed, soaked in sterile distilled water for 3 h, and autoclaved in batches of 40 in foil-covered plastic beakers at 121 °C for 45 min. The blocks were placed into 2-week-old cultures of *H. fasciculare* or *P. velutina* grown on 500 ml MA in 2-litre wide-necked flasks and the flasks incubated for 5 or 15 weeks at 20 °C before removal of the wood blocks as inocula. Blocks incubated for 15 weeks were cut before use into 1 cm^3 units.

Experimental procedure. Square (24 × 24 cm) lidded plastic bioassay dishes 2 cm deep (available from Nunc; Gibco, Paisley, UK) were three-quarters filled with a sieved (4 mm mesh size) sandy loam soil collected from Friary Woods, near Bath (ST 785588). Autoclaved, uncolonized wood blocks ('baits') or washed plastic bottle caps (to serve as controls) were then placed either singly (small baits) or in groups of four (large baits) into the soil 13 cm diagonally away from one corner of the dish. After 7 d at 20 °C, to permit equilibration of moisture between baits and soil, either 8 cm^3 (large) or 1 cm^3 (small) colonized wood blocks were placed 5 cm away from the baits or controls further along the same diagonal. All experiments were made in triplicate, and the dishes were incubated at 20 °C inside polythene bags in darkness.

Outgrowth from and between the wood blocks was examined at regular intervals (as indicated in Fig. 6), and patterns visible through the underside of the dishes were recorded directly by photocopying on a Xerox 1045 machine. Radial outgrowth was measured along diagonals extending from the corners of inoculum blocks.

RESULTS

Xerox records of representative sets of mycelial outgrowth patterns are shown in Figs 1–5. Combined growth data from all the experiments are recorded graphically in Fig. 6.

Outgrowth patterns in control dishes were similar in all cases, irrespective of species and inoculum size, in that a persistent, symmetric radiating system was established which reached the edge of the dishes after about 25–30 d for *P. velutina* and 50 d for *H. fasciculare* (Fig. 1). The only important deviation from this pattern was the limitation of extension after approximately 35 d by *H. fasciculare* extending from small inoculum blocks (Fig. 6*b*).

By contrast, marked changes in the form and growth characteristics of the mycelium occurred in response to contact with baits, and furthermore these changes were influenced by the relative sizes of inocula and baits, and by the fungal species concerned (Figs 2–5).

Contact with the baits was made by *P. velutina* after 15 d and by *H. fasciculare* after 22 d. The first visible effects of contact, which in some cases appeared to follow curvature of mycelial cords (e.g. Fig. 3), were the production from the cords of fan-shaped systems of effuse mycelium spreading over the baits. Associated with this was a virtually immediate thickening of the connective cords. During the following days there was an obvious cessation of radial extension and eventual regression of the non-connective remainder of the mycelial front of most of the parent colonies (Fig. 6). Two important exceptions to this pattern were seen with *P. velutina*. Firstly, those systems growing from large inocula continued to extend at the same rate as controls (Fig. 6). Secondly, with small inocula after contact had been made with the baits, some cords not initially growing towards the bait continued to extend to the edge of the dish, and then apparently redirected towards the bait before eventually making contact and becoming firmly established after 70 d (Figs 5*d–f* and 6*d*).

Cessation of extension in the case of *H. fasciculare* grown from large inocula was clearly associated with a change from a regular colony margin (Fig. 2*c*) to an irregular colony margin (Fig. 2*d*).

Regression of the non-connective parent colony mycelium was also strongly correlated with emergence of new outgrowth from the baits (Fig. 6). In *H. fasciculare* this renewed outgrowth was in the form of fan-shaped sectors of mycelium, and was always away from the inoculum block, hence conserving the polarity of the original system. There was less regression of mycelia from large inoculum blocks than from small ones: here numerous anastomoses were visible

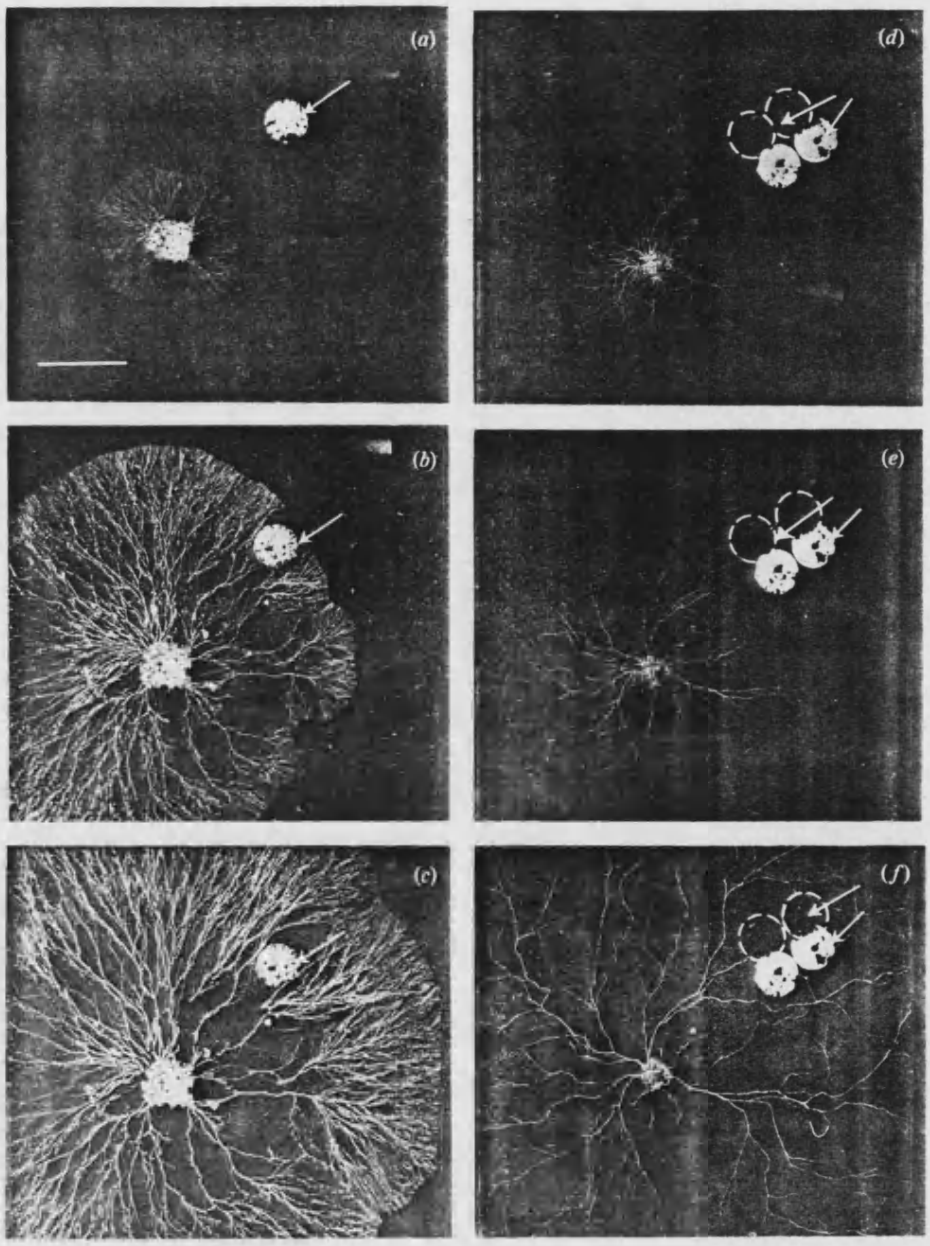

Fig. 1. Outgrowth patterns in control dishes of *H. fasciculare* (*a–c*) after (*a*) 20 d; (*b*) 51 d; (*c*) 68 d; and *P. velutina* (*d–f*), after (*d*) 8 d; (*e*) 12 d; (*f*) 44 d. Bar represents 4 cm. Arrows indicate position of single bottle caps in (*a–c*) and groups of four (some obscured by soil) in (*d–f*). In this and following figures (i.e. Figs 2–5) the left- and right-hand margins of the photographs coincide with, or extend slightly beyond, the edge of the dishes.

2*e*, *f*). Outgrowth of *P. velutina* from large baits (hence small inocula) was similar to that seen with *H. fasciculare* except that the fans of *P. velutina* were more diffuse and showed rather less polarity (Fig. 5). However, outgrowth from small baits (hence large inocula) was by contrast minimal and merged directly with mycelium emanating from inocula, which, as mentioned previously, had shown no signs of cessation of extension or regression (Fig. 3).

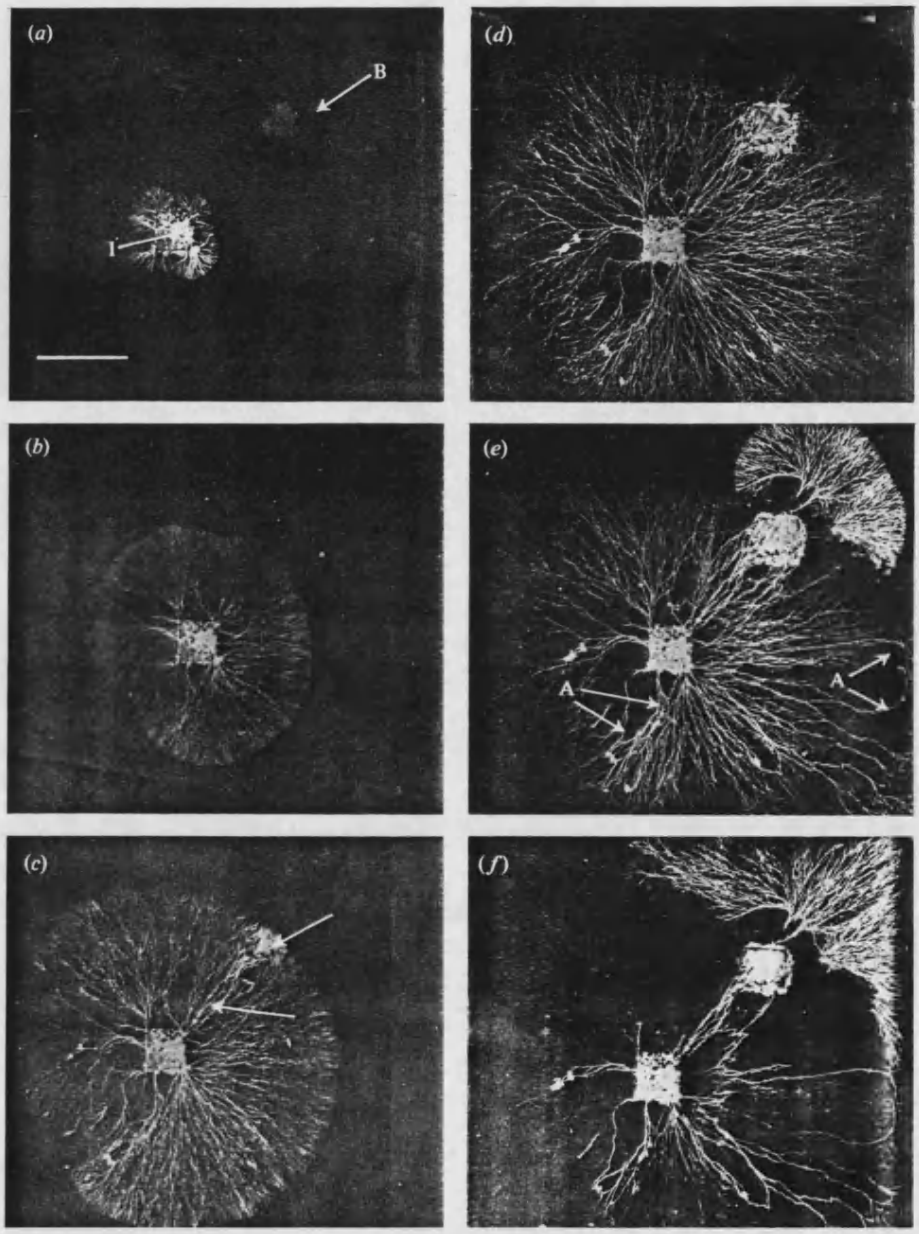

Fig. 2. Outgrowth pattern of *H. fasciculare* from a large inoculum (I) and its response to contact with an equally sized bait (B) after (*a*) 8 d, (*b*) 20 d, (*c*) 31 d, showing the regular margin present on contact with the bait, and thickening of connective cords (arrowed); (*d*) 51 d, showing irregular morphology at the edge of the mycelium; (*e*) 68 d, showing outgrowth from the bait, regression of non-connective mycelium, and position of anastomoses (A) between cords; (*f*) 85 d. Bar represents 4 cm.

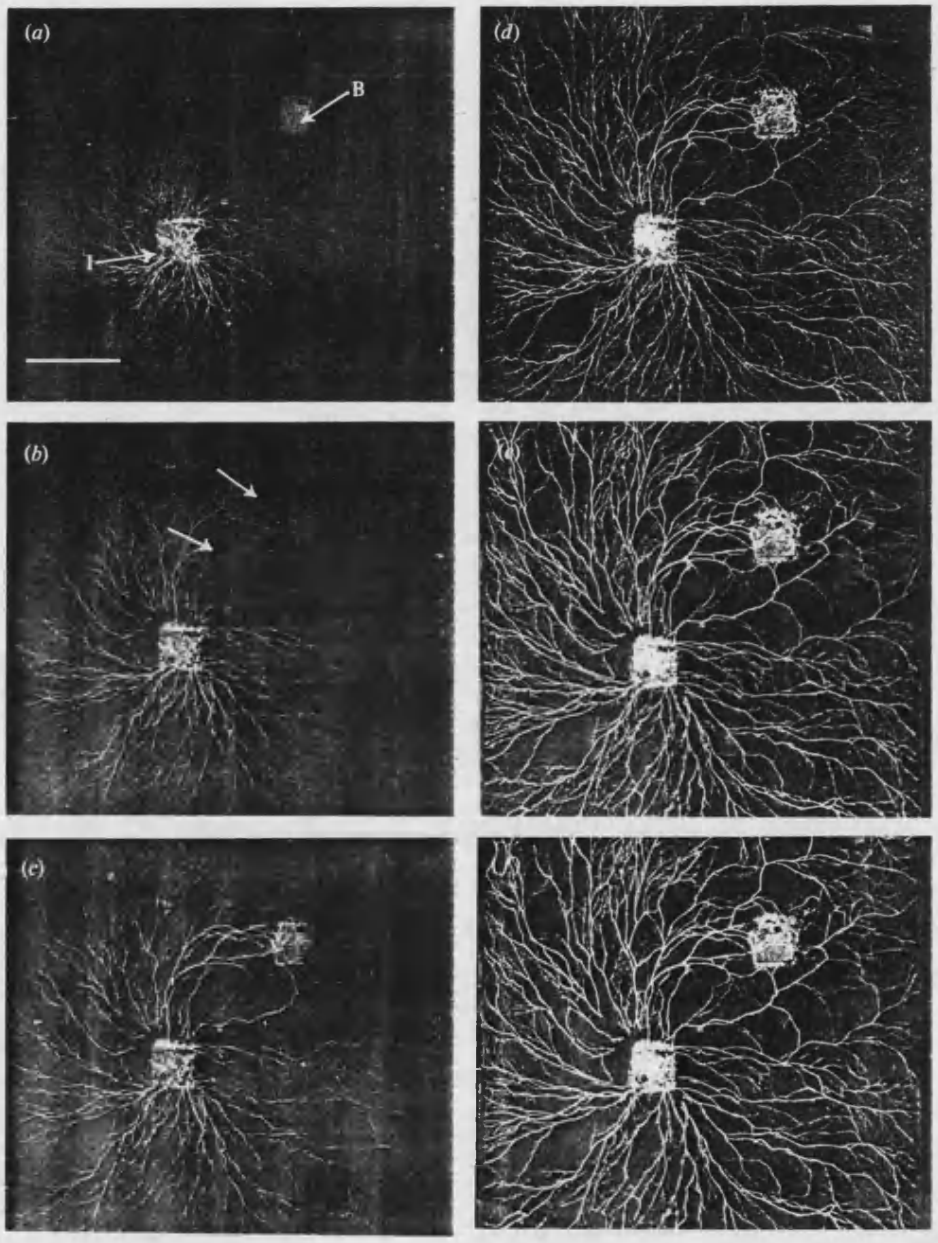

Fig. 3. Outgrowth pattern of *P. velutina* from a large inoculum (I) and its response to contact with an equally sized bait (B) after (*a*) 12 d; (*b*) 20 d, arrows indicate curvature of cords towards the bait; (*c*) 26 d; (*d*) 44 d; (*e*) 62 d; (*f*) 77 d, showing no regression of non-connective mycelium. Bar represents 4 cm.

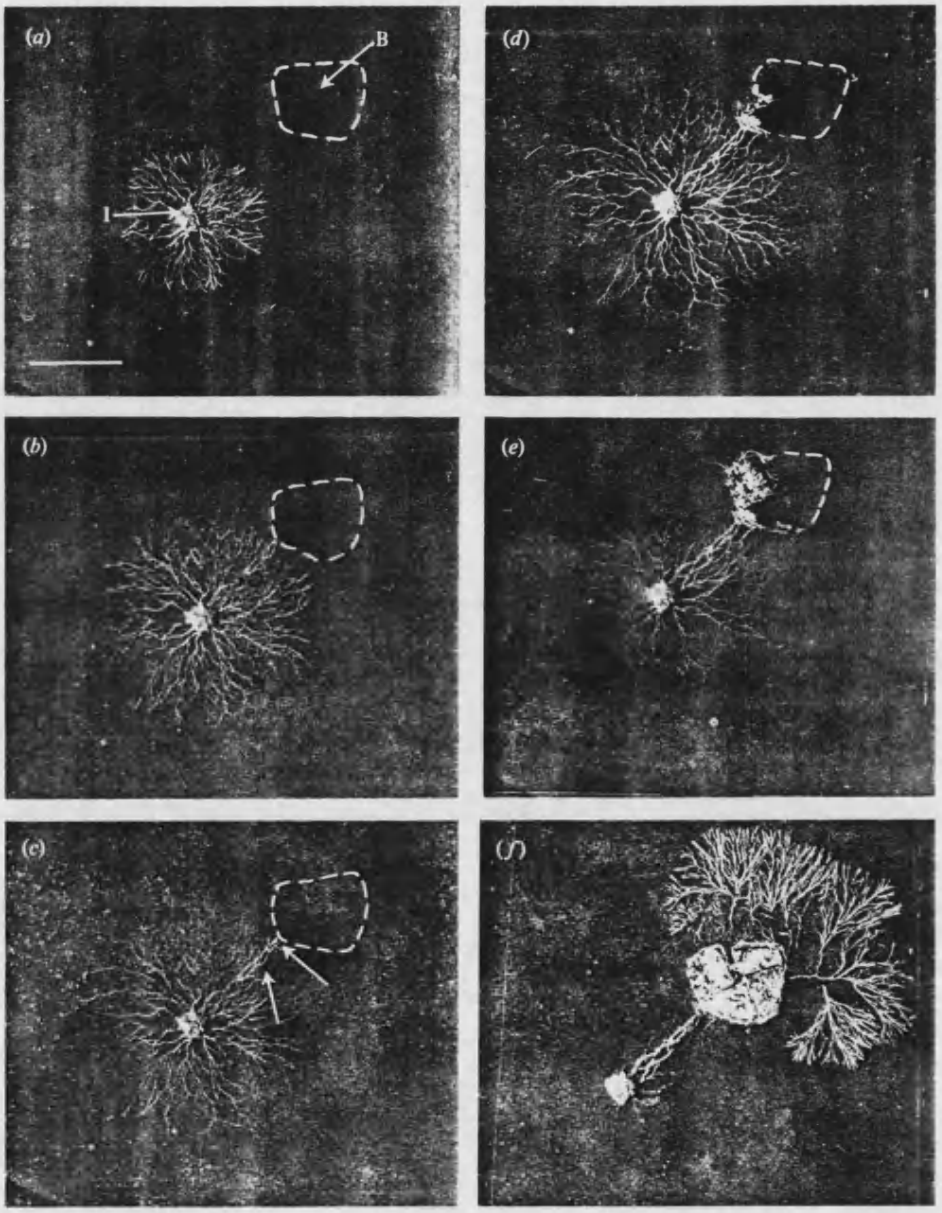

Fig. 4. Outgrowth pattern of *H. fasciculare* from a small inoculum (I) and its response to contact with a large bait (B) after (*a*) 12 d; (*b*) 19 d; (*c*) 22 d, showing effuse mycelium produced on contact with the bait and thickened connective cord (arrowed); (*d*) 27 d; (*e*) 44 d, showing regression of non-connective mycelium; (*f*) 70 d, showing outgrowth of mycelium from bait. Bar represents 4 cm.

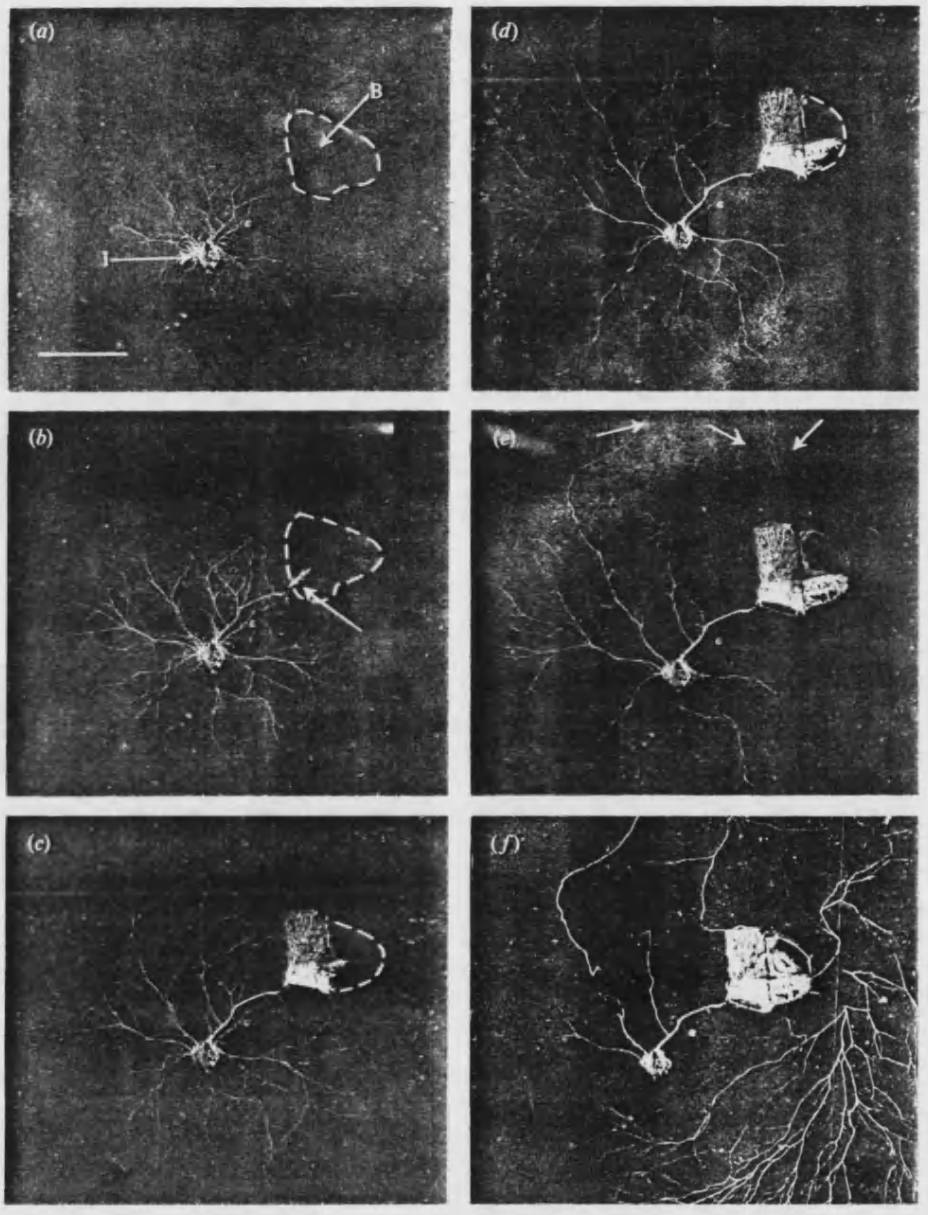

Fig 5. Outgrowth pattern of *P. velutina* from a small inoculum (I) and its response to contact with a large bait (B) after (*a*) 8 d; (*b*) 12 d, showing effuse mycelium at the point of contact with bait (arrowed); (*c*) 22 d, showing thickened connective cord; (*d*) 27 d; (*e*) 44 d, showing curvature and redirected growth of a non-connective cord towards bait (arrowed); (*f*) 70 d, showing arrival of redirected cord, regression of other non-connective cords and outgrowth from the bait. Bar represents 4 cm.

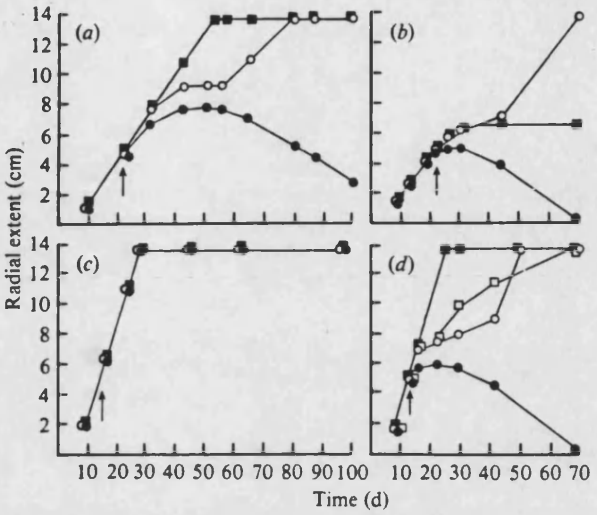

Fig. 6. Collected mean data showing outgrowth of *H. fasciculare* (*a*, *b*) and *P. velutina* (*c*, *d*) from large (*a*, *c*) and small (*b*, *d*) inocula along the diagonal between inoculum and baits (○), non-connective mycelium (●), cords which eventually become connective only after 70 d (Fig. 5*e*, *f*) (□) and controls (■). Error bars are omitted for clarity, but variation between replicates was less than 4%. Arrows indicate time of contact with the bait.

DISCUSSION

The present observations demonstrate the strikingly different properties of mycelia growing on discontinuously supplied resource units as opposed to under homogeneous conditions. Essentially, these properties may be related to the distinctive demands on the one hand for efficient discovery of nutrient depots and on the other for economical usage and redistribution of resources available to a mycelium interconnecting different depots.

Two types of behaviour may facilitate efficient discovery of nutrient depots (Rayner *et al.*, 1985). The first involves production of sparse exploratory mycelial systems which only develop further once contact with a suitable nutrient base has been made. Evidence for this type of behaviour has been provided by the present study and also for certain cord-forming mycorrhizal fungi by Read (1984). The underlying mechanism is obscure, but may involve establishment of nutrient exchange between food bases: indeed Watkinson (1975) has used the establishment of cords of *Serpula lacrimans* between separated nutrient agar plugs as a basis for translocation studies.

Secondly, direct growth of hyphae, mycelium or mycelial aggregates may occur towards nutrient sources. Until recently, evidence for such directed growth has mostly been confined to certain water moulds (*Saprolegniaceae*) (Fischer & Werner, 1958), but there are now indications of similar mechanisms in some wood-inhabiting fungi (Mowe *et al.*, 1983; Thompson & Rayner, 1983; Thompson, 1984).

In the present study fairly marked curvature or redirection of connective cords towards the baits was apparent in several cases. However, this was not a consistent observation, and the experiments were also not designed specifically to distinguish between causative factors, be these release of a soluble or a volatile compound from the blocks, or some other local change in microenvironmental conditions. The controls were, however, intended to simulate effects of a discontinuity in the soil system, and the experimental design should also have minimized development of moisure gradients. We have obtained better evidence for directed growth by *H. fasciculare* and *P. velutina* by growing systems over moist, compacted sand, rather than through soil (data not shown).

Evidence for economic usage and redistribution of resources by mycelium interconnecting resource units was provided by the inhibitory effects of contact with baits on the remainder of the colony margin, by the regression of mycelium originating from the inoculum associated with renewed growth from the bait, and by the differences between outgrowth patterns, particularly by *P. velutina*, using large and small inocula/baits. With respect to the last point, it would obviously be efficient in growth from a small to a large base to reallocate resources to the latter, but not vice versa: this was brought out by the maintenance of the mycelial systems emanating from large inocula, but regression of these from small inocula. It should also be noted that the small inocula used in the present experiments would contain a smaller nutrient supply not only in relation to their volume, but also because they had been incubated with mycelium for a longer time period.

In summary, these observations provide evidence for the corporate nature of mycelial systems and how this facilitates migration between nutrient depots. Analogies with other spatially indeterminate eukaryotic bodies such as stoloniferous plants and myxomycete plasmodia are strong and we think that recognition of this fact could provide a useful basis for understanding fungal behaviour and a fresh impetus for ecological and physiological studies.

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CHAPTER 6.

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Foraging patterns of *Phallus impudicus*, *Phanerochaete laevis* and *Steccherinum fimbriatum* between discontinuous resource units in soil

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Key words: Basidiomycetes; Foraging; Fungal growth; Mycelial cord; Wood decay

1. SUMMARY

Wood blocks colonised by the basidiomycetes *Phallus impudicus, Phanerochaete laevis* and *Steccherinum fimbriatum* were placed individually in plastic trays containing moist, unsterilised soil. All three fungi grew out radially from the inoculum blocks, forming networks of mycelial cords. Outgrowth patterns of *P. impudicus* and *P. laevis* were similar in controls to those in experiments where a second uncolonised wood block was placed as a 'bait' several centimetres away from the inoculum block. However, contact with the bait by *S. fimbriatum* resulted in marked changes in growth pattern. These changes included cessation of radial extension from the inoculum, thickening of connective mycelium between inoculum and bait, outgrowth from the bait in the original direction of travel and regression of non-connective mycelium. These observations emphasize the collective organisation of mycelial systems and the differences in their growth pattern which can arise from varying foraging strategies.

2. INTRODUCTION

The many studies which have been made of fungal growth under homogeneous conditions of nutrient supply are uninformative about growth patterns under circumstances, common in nature, where nutrients are heterogeneously and discontinuously distributed. Examples of such patterns are provided by certain decomposer basidiomycetes inhabiting the woodland floor which form networks of mycelial cords, often many metres in total length, interconnecting pieces of decaying wood and litter [1–3]. Recent experimental studies with two of these fungi, *Hypholoma fasciculare* and *Phanerochaete velutina*, have produced evidence of powerful mechanisms which increase the efficiency of growth between discontinuous nutrient depots [4–6]. In this paper we report on an extension of these studies to three other species, *P. impudicus, P. laevis* and *S. fimbriatum* and

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discuss how the different outgrowth patterns exhibited by these fungi may be explained in terms of variations in foraging strategy.

3. METHODS

3.1. Strains and culture media

P. impudicus was isolated from a mycelial cord attached to a sporophore collected from mixed woodland at Home Covert, Devizes, Wilts., U.K. (N.G. Ref. SU008631). *P. laevis* and *S. fimbriatum* were isolated from decayed beech (*Fagus sylvatica*) wood collected from Farleigh Hungerford Woods, Wilts., U.K. (ST795563). All species were routinely cultured on 2% (w/v) malt extract agar (MA; 20 g Munton & Fison spray malt A, 15 g Lab M agar no. 2. l^{-1}).

3.2. Preparation of wood block inocula and baits

Wood blocks approximately 8 cm^3 were cut from freshly felled beech trees and stored at

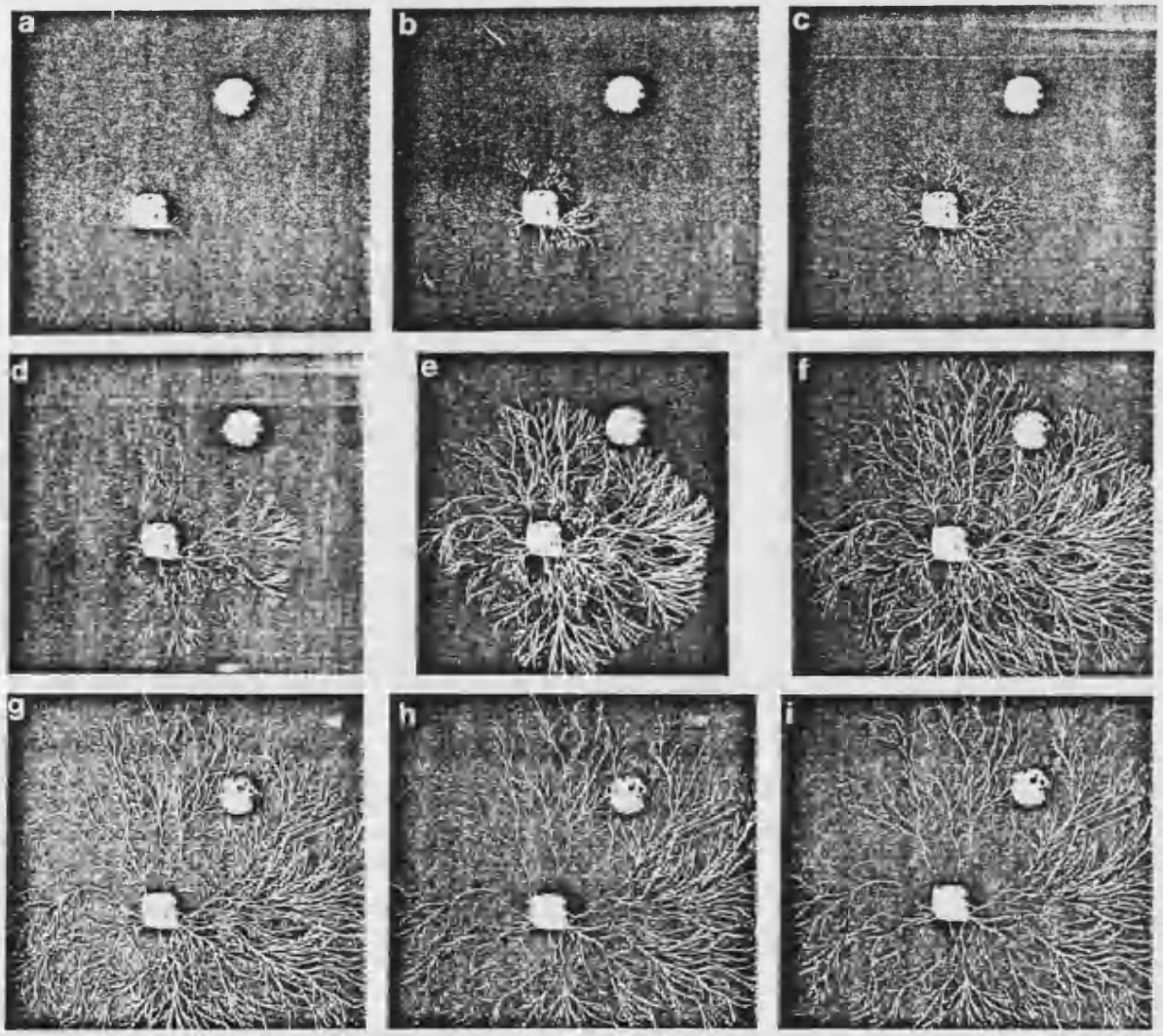

Fig. 1. Outgrowth pattern of *Steccherinum fimbriatum* in a control dish after (a) 7 d; (b) 12 d; (c) 20 d; (d) 26 d; (e) 31 d; (f) 45 d; (g) 63 d; (h) 78 d; (i) 90 d. Wood block 2×2 cm square.

−18°C. Before use the blocks were thawed, soaked in sterile distilled water for 3 h and autoclaved in batches of forty in foil-covered beakers at 121°C for 45 min. Those to be used as inocula were then placed onto 2-week-old cultures of *P. impudicus*, *P. laevis* and *S. fimbriatum* grown on 500 ml MA in 2 l conical flasks, and incubated for 5 weeks at 20°C before removal for use in experiments.

3.3. Experimental procedure

Square, lidded plastic bioassay dishes, 24 × 24 × 2 cm (available from Nunc: Gibco, Paisley, U.K.) were three-quarters filled with a sieved (4 mm mesh size) sandy loam soil with a moisture content of about 37% by dry weight (i.e., field capacity). Autoclaved, uncolonised wood blocks ('baits') or washed plastic bottle caps (to serve as controls) were then placed into the soil 13 cm diagonally away from one corner of the dish. After 7 days at 20°C, to permit equilibration of moisture between baits and soil, inoculum blocks were placed 3 cm away from the baits or controls further along the same diagonal. All experiments were made in triplicate, the plates being incubated inside polythene bags in darkness at 20°C.

Outgrowth patterns from and between the wood blocks were examined at regular intervals (as indicated in Fig. 2) and patterns visible through the underside of the dishes were recorded directly by photocopying on a Xerox 1045 machine. Radial outgrowth was measured along diagonals extending from the corners of the inoculum blocks.

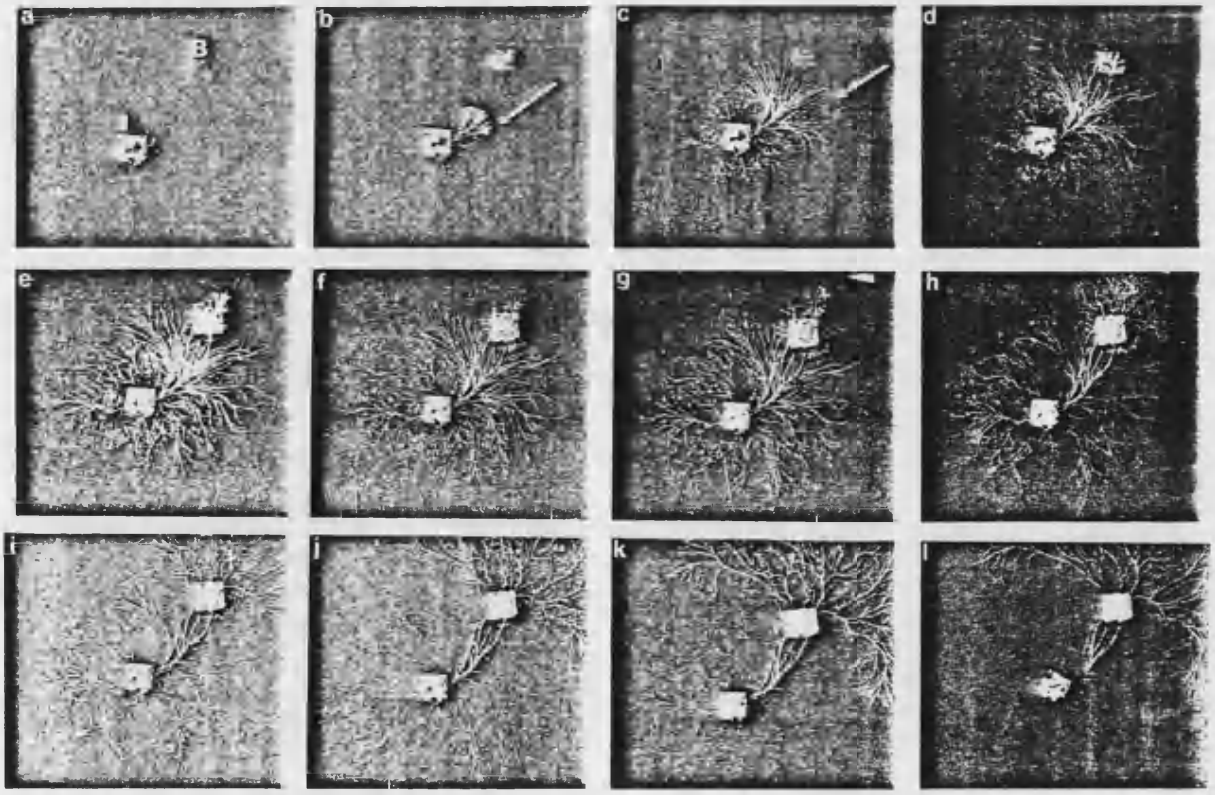

Fig. 2. Outgrowth pattern of *Steccherinum fimbriatum* from an inoculum wood block (I) and colonisation of an unoccupied bait wood block (B) after (a) 7d; (b) 12 d; (c) 20 d; (d) 26 d; (e) 31 d; (f) 45 d; (g) 52 d; (h) 63 d; (i) 69 d; (j) 78 d; (k) 90 d; (l) 94 d. Wood blocks 2×2 cm square. Arrows indicate a fast-effuse sector.

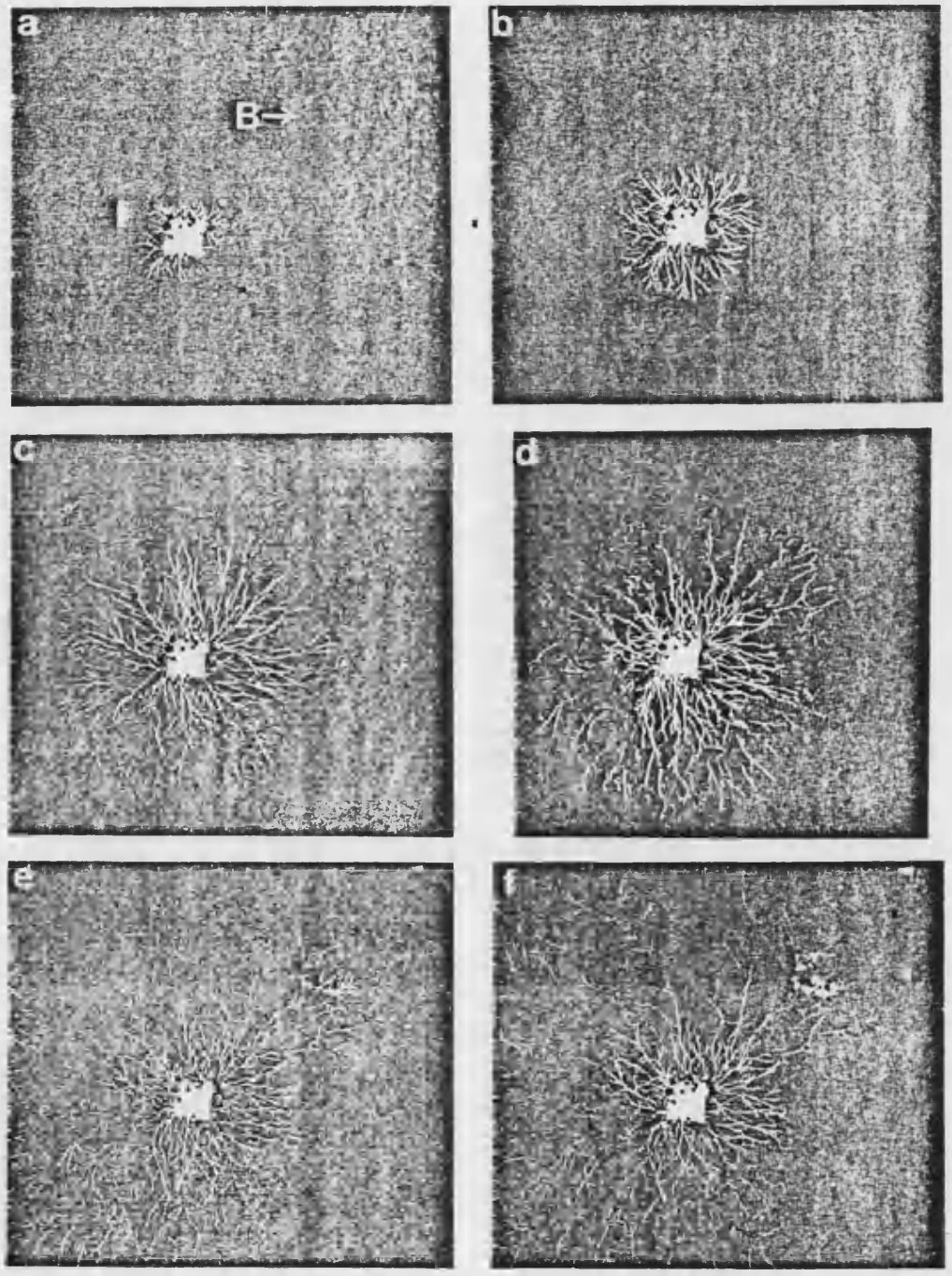

Fig. 3. Outgrowth pattern of *Phallus impudicus* from an inoculum wood block (I) and colonisation of an unoccupied bait wood block (B) after (a) 7d; (b) 12 d; (c) 26 d; (d) 31 d; (e) 63 d; (f) 78 d. Wood blocks 2×2 cm square.

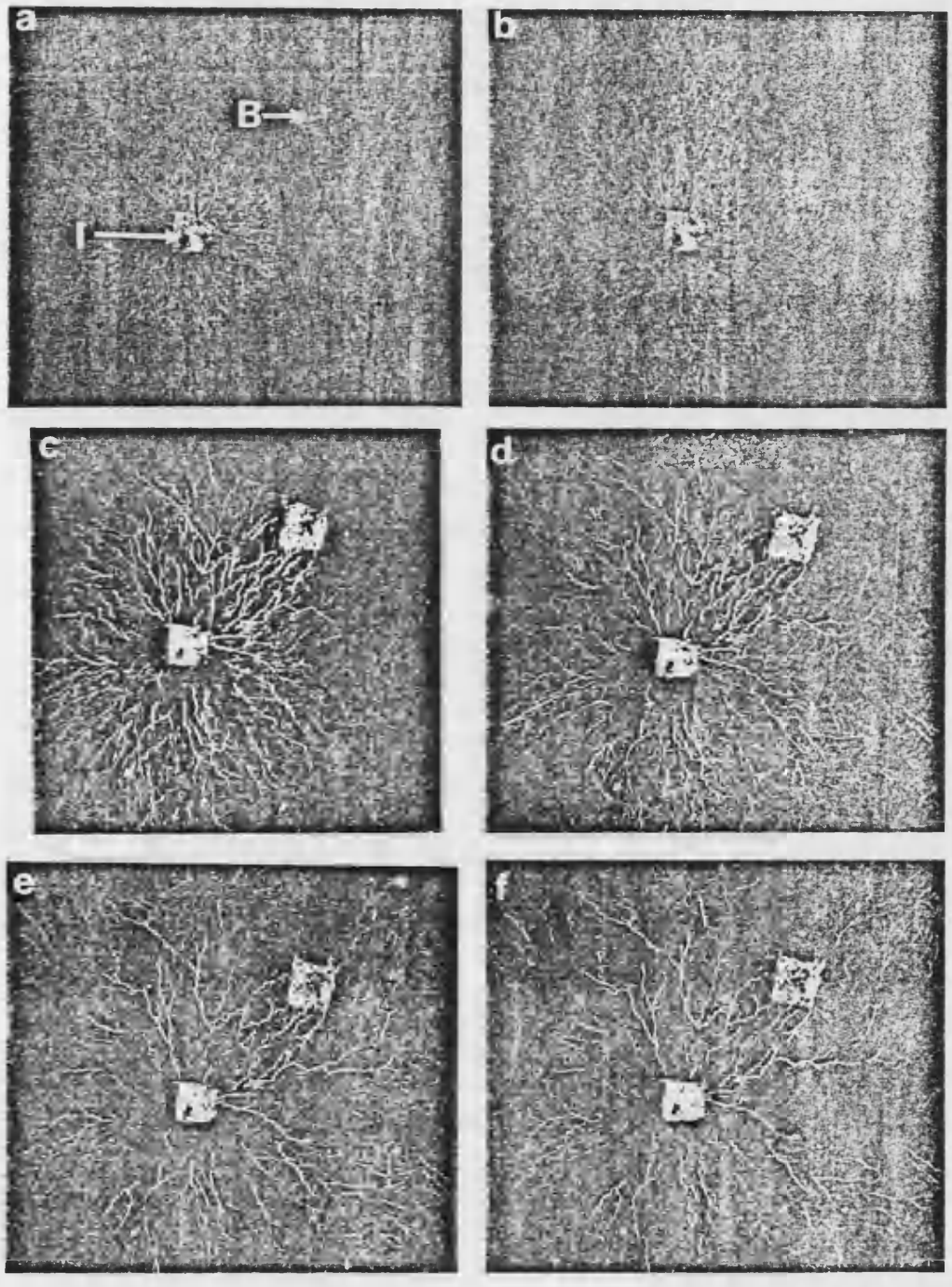

Fig. 4. Outgrowth pattern of *Phanerochaete laevis* from an inoculum wood block (I) and colonisation of an occupied bait wood block (B) after (a) 12 d; (b) 20 d; (c) 31 d; (d) 45 d; (e) 78 d; (f) 90 d. Wood blocks 2 × 2 cm square.

4. RESULTS

Xerox records of mycelial outgrowth patterns in individual dishes are shown in Figs. 1–4. Aggregate data for mycelial extension from these and all other plates are shown graphically in Fig. 5.

Similar outgrowth patterns were produced in control dishes by all three species, resulting in persistent, radially symmetrical systems which reached the edge of the dishes after 70 days with *P. impudicus* and *S. fimbriatum* (Fig. 1) and 50 days with *P. laevis*.

The patterns produced by *S. fimbriatum* in dishes to which uncolonised baits had been added (Figs. 2, 5c) differed radically from those in control dishes. Contact with baits after 20 days resulted in the production of an effuse mycelium which spread over the baits concomitant with marked thickening of connective cords. Outgrowth from the baits followed by extension of non-connective mycelium slowed considerably and totally ceased after 50–70 days and was accompanied by regression of most of the non-connective mycelium. This new growth was directed away from, but was morphologically similar to, the original outgrowth. The position of the distinct, rapidly extending sector in the initial outgrowth towards the bait illustrated in Fig. 2 may have been fortuitous, since the occurrence and location of these sectors varied between different dishes. Outgrowth systems entirely lacking such sectors produced similar responses following contact with baits.

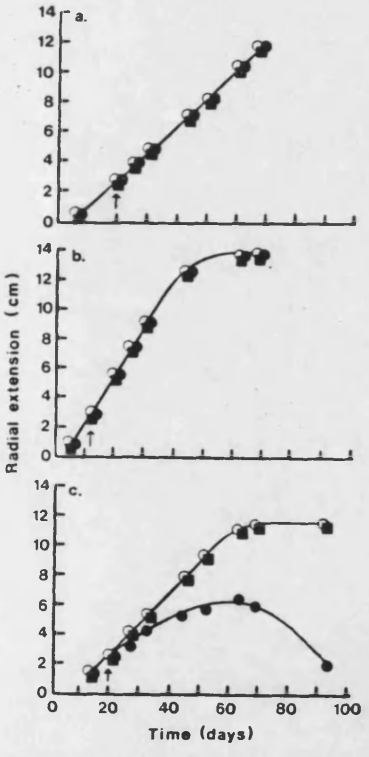

Fig. 5. Mean data (three replicates) showing outgrowth of *Phallus impudicus* (a), *Phanerochaete laevis* (b) and *Stecchérinum fimbriatum* (c) from inocula along the diagonal between inoculum and baits (○), non-connective mycelium (●) and controls (■). Error bars are omitted for clarity, but variation was less than 8%. Arrows indicate time of contact with the bait.

Unlike *S. fimbriatum*, contact with baits by *P. impudicus* (Figs. 3, 5a) after 20 days and *P. laevis* (Figs. 4, 5b) after 12 days did not result in any marked deviation in growth pattern from that observed in controls. Despite the production of thick, apically dominant (rhizomorphic) cords by *P. impudicus* from the inocula, colonisation of the baits was poor compared with *P. laevis* and *S. fimbriatum*, generally being restricted to the block periphery. In one replicate where colonisation of the baits was best, a limited region of mycelial regression was apparent in mycelium adjacent to connective cords (Fig. 3e, f).

5. DISCUSSION

Added to our previous study with *H. fasciculare* and *P. velutina* [4], the results of the present investigation illustrate the collective organisation and functioning of mycelial cord systems and the way in which different degrees of coordination between individual hyphal 'search' units produce a range of distinctive growth patterns. These patterns maximise the efficient discovery of nutrient depots by which a genetic individual extends its domain with minimal expenditure of energy, and can readily be understood in terms of 'foraging theory' as originally developed for animals [7] and recently extended to stoloniferous plants [8].

Given that microenvironmental conditions and physiological properties (e.g., ability to translocate organic nutrients) allow mycelial outgrowth into soil, a variety of interconnected options arises. These can be understood in terms of the following series of questions. (1) Should commitment of biomass to outgrowth be on a small or a large scale? (2) Should outgrowth be densely branched and slow to extend, or sparsely branched with a more rapid extension rate? (3) Should hyphal search units fan out over a broad front or be focussed along particular paths by apical dominance? (4) Should individual search units be dependent on one another or be independent?

The pattern and responsiveness of the foraging systems, resulting from varying foraging strategies, will depend on which options are most cost effective in terms of energy gain and expenditure. Cost effectiveness is conditioned by the probability in space and time of encountering colonisable nutrient depots within range of an existing base. This probability depends in turn on how narrowly or widely set is the organism's tolerance of microenvironmental conditions both in terms of stress factors (stress tolerance) and the ability to defend or wrest domain from competitors (defensive or attacking combative ability) [9].

The most striking changes in growth pattern following contact with the baits in the present and previous study [4] were produced by *H. fasciculare* and *S. fimbriatum*. Such behaviour suggests dependence on short-range foraging, associated with unspecialised environmental requirements and/or high attacking combative ability when present as substantial biomass. Although *H. fasciculare* and *S. fimbriatum* exhibited superficially similar patterns of regression and outgrowth following contact with the baits, some important details differed, notably *S. fimbriatum* exhibited a switch between slow-dense and fast-effuse outgrowth patterns, and also maintained extension rate following contact with baits. These differences may be connected with adaptation by *S. fimbriatum* to growth in soils with fluctuating moisture regimes and the slower decay rate of wood caused by this species [10]. This slower decay rate is probably associated with production of persistent mycelial cord systems interconnecting variably sized woody resource units (cf. *P. impudicus* below; *H. fasciculare* forms less persistent systems and causes rapid decay in large stumps and logs).

P. laevis, *P. velutina* and *P. impudicus* exhibit longer-range foraging patterns, although the last species differs from the first two in producing more rhizomorphic cords and being less effective both as a cause of decay (unpublished data) and as a colonist of baits. It seems likely that the strategy which has evolved in *P. impudicus* is formation of a persistent network of cords from which effective colonisation of suitable substrata can be achieved as soon as these fall within range. *P. laevis* and *P. velutina* on the other hand are more powerful decomposers and possess a higher degree of combative ability, allowing them to colonise available resources from sparse biomass even when these are occupied by other fungi. Their sparser cord systems are relatively independent, so that re-allocation to successful lines from unsuccessful search paths does not occur readily. However, here it is of considerable significance that a greater degree of re-allocation can be induced in *P. velutina* by greatly increasing the size of baits relative to inocula [4]. The same may well apply to *P. laevis*, but this possibility was not tested during the current investigation.

ACKNOWLEDGEMENTS

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ESTABLISHMENT STRATEGIES OF SOME DECOMPOSER BASIDIOMYCETES

PART II

CHAPTER 7.

Spatial dynamics and interactions of the woodland fairy ring fungus, *Clitocybe nebularis*

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Key words: ecological strategies, fairy rings, foraging, fungal communities, mycelial development.

SUMMARY

The extension rates of *Clitocybe nebularis* (Batsch ex Fr.) Kummer strains on 2 % malt agar were only 30-40 % of those, up to 3.4 mm d^{-1}, observed in woodland at equivalent exponential mean temperatures. Extension of mature field systems was accomplished by mycelial annuli or arcs 30-40 cm wide, differentiated into a leading edge of mycelial cords followed by a zone of dense, diffuse mycelium which bleached litter components, and a trailing edge of greyish, lysed mycelium. Disruption of mature annuli by natural obstacles or experimental re-orientation within the mycelial band resulted in regression of the affected segment of mycelium. Localised lysis following encounter with an obstacle by immature patches of mycelium with a diameter of 30-50 cm, led to polarised development of the residual mycelium.

Strains from different fruit bodies were somatically compatible when paired on 2 % malt agar if sampled from the same ring, but incompatible if from different rings, resulting in mutual antagonism and formation of a persistent demarcation zone. By contrast, collision between adjacent systems in woodland culminated in mutual obliteration of the interaction fronts. *C. nebularis* was non-combative when paired against other decomposer basidiomycetes on 2 % malt agar, being either replaced or deadlocked but not replacing mycelia of these fungi.

The implications of these observations are discussed in terms of emerging concepts of ecological strategies, foraging theory and polarity in mycelial collectives.

INTRODUCTION

Although typified by one species, *Marasmius oreades* (Bolt. ex Fr.) Fr., 'fairy rings' are formed by many fungi with diverse nutritional modes. Whether these fungi are biotrophic (e.g. Ford, Mason & Pelham, 1980), necrotrophic (e.g. Filer, 1965) or saprotrophic (e.g. Weaver, 1975) in their nutrition, their common characteristic, an annular mycelial growth zone encompassing an empty centre, has long eluded complete explanation. Two recurrent hypotheses involve the production of autoinhibitory compounds and depletion of nutrients in the central regions (Shantz & Peimeisel, 1917; Wollaston, 1807; Bayliss, 1911; Weaver, 1975; Smith & Rupps, 1978), but neither has been satisfactorily tested.

However, the availability of nutrient resources in advance of the growth fronts does appear to be implicit in continued extension, and the fronts are commonly reported to regress when encountering obstacles or barren terrain (Shantz & Piemeisel, 1917; Parker-Rhodes, 1955; Smith, 1957, 1980; Ingold, 1974; Weaver, 1975; Stevenson & Thompson, 1976; Fisher, 1977). Moreover, the collision of two fronts has been reported to culminate in mutual extinction (Wollaston, 1807; Shantz & Piemeisel, 1917; Parker-Rhodes, 1955; Smith, 1980), a situation which appears to contrast with observations of somatic incompatibility between fungal genotypes in culture or in substrata, such as wood, with a finite boundary. In the latter case, persistent demarcation zones due to mutual antagonism are maintained between adjacent mycelia (e.g. Rayner *et al.*, 1984).

Many of the studies, cited above, of spatial dynamics of fairy

rings, have concerned grassland fungi. Fewer reports have been made on woodland fairy rings, where, in addition, an important distinction has not always been made between 'free' rings of saprotrophic, decomposer fungi and 'tethered' rings of ectomycorrhizal species confined to host roots (Ford *et al.*, 1980; Gregory, 1982). In this paper we report on the spatial dynamics of experimentally disturbed and undisturbed rings formed by the common woodland decomposer, *Clitocybe nebularis* (Batsch ex Fr.) Kummer and compare the field observations with mycelial growth and interaction data from laboratory studies. We go on to discuss a view of fairy rings as foraging mycelial collectives whose developmental polarity is determined by their different ecological strategies with respect to patterns of nutrient capture and turnover and response to competitors.

MATERIALS AND METHODS

Field site and monitoring of extension patterns and environmental fluctuations

Annuli, arcs or patches of fruit bodies of *C. nebularis* were detected, during October 1983, in a deciduous woodland site (dominated by *Fagus sylvatica* L.) at Friary Woods, near Bath (N.G. Ref. ST785588). Leaf litter was carefully removed to expose the underlying mycelia and canes placed at 0.1-1 m intervals to mark the growth fronts of six mature, intact or disrupted annuli, and two 'immature' patches of mycelium. The litter was then replaced. Subsequent extension until September 1984 was measured by re-excavating and marking the mycelium at each cane at 4-8 week intervals. Gross mycelial morphology was observed by further

excavation of litter, and by examination under a dissecting microscope of thin radial sections cut across the mycelium to a depth of 10 cm below the litter surface into mineral soil.

Temperature at each system was monitored using maximum/minimum thermometers and chemical sensors placed at the soil interface. The chemical sensors involved use of the sucrose inversion technique described by Dowson, Rayner & Boddy (1988a), and exponential mean temperature was calculated using the computer programme of Boddy & Morris (1984).

Precipitation was estimated every 3-6 weeks at fifteen locations throughout the site using 250 ml plastic conical flasks partially buried and fitted with 9 cm diameter funnels held in place by rubber bungs.

Soil moisture was recorded as matric potential using the modification of the filter paper technique (Fawcett & Collis-George, 1967) described by Dowson *et al.* (1988a).

Experimental re-arrangement of rings

Sods of earth, litter and mycelium 0.6 x 0.6 wide and 0.1 m deep were cut from three of the nature systems during August 1985, lifted with a 0.6 x 0.6 m metal sheet, and each then subjected to one of the following procedures: (1) direct replacement as controls; (2) relocation to a new site several metres in advance of or behind the mycelial front to replace an equivalently sized sod; (3) re-orientation through 180° before direct replacement, so that the trailing edge aligned with the growth front of the pre-existing mycelium; (4) bisection parallel to the growth front, direct replacement of the trailing half and re-orientation

of the front half through 180° prior to direct replacement. All treatments were undertaken in triplicate (i.e. once for each system) and subsequent growth monitored for 6 months.

Growth and interactions on agar plates

Fruit bodies of *C. nebularis* were sampled at 1-2 m intervals from the circumference of the six mature systems and once from each of the patches, and isolates prepared by excising small pieces of tissue and placing them on 2 % malt extract agar (MEA; 20 g Munton & Fison spray malt A, 15 g Lab M agar no. 2 per litre distilled water) sometimes containing 100 ppm novobiocin. The isolates were paired in various combinations amongst themselves and against other decomposer basidiomycetes (see Results; for origin of strains see Dowson, Boddy & Rayner, 1988b) by placing inocula, *ca.* 4 mm diameter and cut from the margin of actively growing colonies, 3 cm apart in the centre of 9 cm non-vented plastic Petri dishes containing 2 % MEA. The dishes were incubated in darkness for up to 12 weeks at 15 or 20 °C.

The radial extension rates in darkness on 2 % MEA of five strains, each representing a different ring, were recorded at 5, 10, 15, 20 and 25 °C by measuring colony increment along two diameters drawn at right-angles on the underside of 9 cm non-vented Petri dishes.

RESULTS

Development and interactions of woodland systems

The mean daily extension rates of the six mature systems during one year are shown in Figure 1, together with microclimatic data.

The average rate of extension throughout the year was 2.3 mm d^{-1}, the minimum 0.5±0.1 mm d^{-1} in winter and the maximum 3.4±0.3 mm d^{-1} during late Spring, Summer and Autumn. Assuming constant growth of 0.9 m y^{-1}, intact annuli of 6 and 8 m radius were estimated to be 7 and 9 years old, and arcs with calculated radii of 10-30 m estimated to be 11-33 years old.

Microclimatic parameters were not usually significantly different between rings ($p \leq 0.05$; t-test) and exponential mean temperature varied by no more than 0.5 °C between sites at each sampling time. There was a clear correlation between extension rate and exponential mean temperature.

Mature arcs and annuli consisted of a radially extending band of mycelium 30-40 cm wide. This band was strongly polarised, being differentiated into three morphologically distinct regions (Figs 2,3). At the leading edge white mycelial cords encroached across the surface of litter components and into mineral soil up to 6 cm in advance of a central region of dense, white mycelium which ramified amongst bleaching litter components but did not penetrate into mineral soil. Fruit bodies were produced approximately in the centre of this dense region. At the trailing edge, the mycelium became greyish and progressively fragmented before disappearing altogether, leaving only bleached litter as evidence of its former presence.

By contrast, the two 'immature' systems consisted not of arcs or annuli, but rather of patches of dense, white mycelium 30-50 cm in diameter, with cords at the periphery. The extension rate

of these systems was irregular, but maximum rates were similar to those of mature systems.

The collision of mature arcs or rings resulted in the loss of cords, followed in turn by confrontation of the dense mycelial bands and mutual loss of interfacial mycelium. Cords were also lost following cessation of extension at a bare patch of soil or following contact with large tree roots or stones. Where such obstructions were large enough, ≥0.5 m wide, this resulted in a permanent break in the growth front of mature systems as adjacent mycelium continued to extend, there being only limited lateral growth from the leading edge of the disrupted arc or annulus. In such cases the restricted segments of mycelium lost viability. In the case of immature systems, mycelial lysis initiated at the site of contact with natural obstacles appeared to induce polarisation and the emergence of mycelia differentiated into the three zones characteristic of mature systems.

Effects of mycelial re-arrangements

Mycelium in control sods, cut from rings and replaced directly without re-orientation, continued to extend in alignment with adjacent uncut sections. Mycelia in sods replanted several metres in advance of or behind the ring continued to extend with maintained polarity and limited lateral growth. Mycelia in sods re-oriented through 180° before replacement in the ring gradually disappeared, leaving a permanent gap. A similar outcome occurred when only the front half was re-oriented before replacement: both the front and rear portions disappeared, leaving a gap in the ring.

Growth and interactions on agar

The mean extension rates (mm d^{-1} and 95 % confidence limits) on 2 % MEA of the five strains were as follows: at 5 °C, 0.2±0.2; at 10 °C, 0.8±0.2; at 15 °C, 1.5±0.1; at 20 °C, 1.9±0.2; at 25 °C, 2.1±0.3. Athough the extension rates of isolates from within or between rings were not significantly different ($p \leq 0.05$; t-test), the morphology of strains from different rings varied in production of aerial mycelium and a lobed or even colony margin.

Regardless of the number involved and the distance apart from which they were sampled, isolates from the same ring were always somatically compatible, their mycelia intermingling to produce a uniform mat. By contrast interactions between isolates from different rings were invariably somatically incompatible, resulting in formation of a demarcation zone containing lysed mycelium between the colonies.

Interactions between *C. nebularis* and other decomposer species (see also Dowson *et al.*, 1988b) resulted either in its replacement (R) or deadlock (D) as follows: *Clitocybe flaccida* (Sow. ex Fr.) Kummer (D); *Collybia butyracea* (Bull. ex Fr.) Kummer (D); *Collybia confluens* (Pers. ex Fr.) Kummer (R); *Collybia dryophila* (Bull. ex Fr.) (R); *Collybia maculata* (Alb. & Schw. ex Fr.) Kummer (D); *Collybia peronata* (Bolt. ex Fr.) Kummer (R); *Hypholoma fasciculare* (Huds. ex Fr.) Kummer (R); *Phallus impudicus* (L.) Pers. (R); *Phanerochaete velutina* (DC ex Pers.) Parmasto (R); *Tricholomopsis platyphylla* (Pers. ex Fr.) Sing. (R); *Psathyrella candolleana* (Fr.) Maire (D).

DISCUSSION

Cooke & Rayner (1984) found the existing explanations for fairy ring formation, that is autoinhibition and nutrient depletion, unsatisfactory for woodland systems in view of the leaching effects of rainfall and replenishment of nutrient resources via annual litter fall. Consequently, they considered that an alternative explanation might be found in the high degree of *polarity* of fairy ring development which would occur if the growth front acted as a powerful sink for resources supplied from degenerating mycelium at the trailing edge.

Some support for these concepts of polarity and the establishment of source-sink relationships within natural mycelial systems has come from studies of mycelial cord-forming wood decomposer basidiomycetes growing between discontinuously supplied nutrient depots in soil (Dowson, Rayner & Boddy, 1986,1988a,b; Rayner, Boddy & Dowson, 1987). These studies highlighted the nature of mycelia as coordinated collectives, with varying patterns of interaction between exploratory and exploitative developmental phases. Following the recent upsurge of interest in foraging theory (Stephens & Krebs, 1986; Sutherland, 1987), these patterns were attributed to a range of foraging strategies dictated by the probability of locating colonisable resources within long or short range of existing depots.

In the case of short-range foraging, exemplified by *Hypholoma fasciculare* , dramatic changes in morphogenesis followed arrival of an initially radiate mycelial system at a new

depot. These incorporated consolidation of mycelium connected to the new depot, concurrent with inhibition of extension and subsequent regression of non-connective exploratory mycelium. Regression of the non-connective mycelium was, in turn, associated with strongly polarised outgrowth from the new depot.

These features are strongly reminiscent of the behaviour of fairy rings of *C. nebularis* . Thus the mycelial cords at the growth front can be viewed as foraging parties, dependent for their extension on resources channeled from the exploitative mycelium which continually regresses at its trailing edge where demand exceeds supply. If the cords enter nutritionally rich domain, they will rapidly be superseded by exploitative mycelium. Here they parallel the arrival of mycelial cords of *H. fasciculare* at a nutrient depot. However, if they encounter an obstacle or nutritionally poor region which they cannot traverse, their extension will be inhibited and they will eventually regress as their neighbours progress, in the same way as do non-connective cords of *H. fasciculare* . In these terms, whilst *H. fasciculare* forages in a nutritionally poor matrix with patches of nutrients, *C. nebularis* forages under the reciprocal circumstance of a nutrient-filled matrix (the litter layer) with non-nutritive patches (tree roots, bare soil, stones etc). The otherwise analogous behaviour exhibited by their foraging systems therefore ends in two contrasting results: the formation of interconnections between depots in the wood decomposer, but the formation of polarised arcs or annuli in the fairy ring former.

Apart from the parallels with *H. fasciculare* , a variety of

observations made in the present study are consistent with the interpretation of *C. nebularis* fairy rings as short-range foraging collectives whose polarity is enhanced by a powerful source-sink relationship between exploitative and explorative mycelium. As well as the differentiation of cords, dense and degenerating mycelial zones, these observations include the behaviour of obstructed and rearranged mycelial systems, the effects of collision between rings, and the differences in growth and interaction pattern between laboratory and field systems.

The significant effects of obstructions to growth were the induction of lysis and re-direction of growth in immature systems, and the generation of persistent gaps in mature systems when the obstacles were broader than the width of the mycelial band. As well as implying redistribution of resources from restricted to unrestricted parts of the mycelium, these observations suggest that lateral extension is subservient to outward extension -characteristic of a polarised system. Similar interpretations arise from the rearrangement experiments: extension continues where polarity is conserved by direct replacement without rearrangement or by replanting to locations without neighbouring mycelium, but is discontinued by re-orientation within existing polarised mycelium which acts as a drain on resources. Incidentally, both the restriction of lateral outgrowth into gaps or following transplantation, and the continued extension following tranplantation to the interior of the ring discount a role for nutrient depletion or inhibitors *per se* in annulus production.

The extinction of the interaction interface following collision between fairy rings is also an expected consequence of strongly polarised extension. Following collision, the resultant loss of polarity of the interfacial mycelium renders it a source for redistribution of resources to the growth fronts outlying the confrontation zone which continue to extend.

This situation contrasts with that in a Petri dish, piece of wood or any other resource with a finite boundary at which extension is terminated, hence ceasing to act as a sink (*cf.* Lysek, 1984), and allowing maintenance of the interaction interface. Moreover, it is important to recall that the width of polarised annuli in the field greatly exceeds the diameter of mycelia in Petri dishes. The latter may not therefore attain dimensions at which they can become differentiated into source and sink regions, quite apart from encountering radically different nutrient and other microenvironmental conditions. The faster extension rates of field than laboratory systems at equivalent temperatures is therefore not surprising. However, here it would be useful to have more data about the extension of field mycelia below the critical diameter of 30-40 cm. Although many small mycelia were detected during the present study, the only ones measured had already partially attained such dimensions: nonetheless their uneven growth and apparent polarisation by obstacles implies the expression, above a threshold size, of a switch from slow-dense to fast-effuse extension/branching patterns. There is growing evidence that a wide variety of mycelial fungi can adjust their growth polarity

by such means (Rayner & Coates, 1987; Rayner *et al.*, 1987).

Using the modification of plant population biological terminology (Kays & Harper, 1974; Harper, 1977) advocated by Brasier & Rayner (1987), the somatic incompatibility data were consistent with the interpretation of each ring as representing a unique genet. This is in turn consistent with previous observations of the genetic stability of fairy rings of *M. oreades* (Burnett & Evans, 1966) and with observations of somatic incompatibility and population structure in a wide range of decomposer basidiomycetes (e.g. Frankland, 1984; Rayner *et al.*, 1984, 1987) as well as some root pathogens (e.g. Korhonen, 1978; Stenlid, 1985) and ectomycorrhizal fungi (Fries, 1988). Quite apart from the expression of somatic incompatibility, the mutual extinction of interaction interfaces between colliding polarised mycelia in the field would prevent fruit bodies of different genets coinciding in the same system. However, here it would have been useful to re-orientate sods excised from a ring directly in advance of the growth front (i.e. equivalent to a self-pairing) to see whether a gap was induced. The disturbance of rings of *M. oreades* has been proposed as a means of 'biological control' based on both inter- and intraspecific antagonism (Smith & Rupps, 1978; Smith, 1980). However, somatic incompatibility appears to have been confused with pre-contact inhibition in that study, and in any case the disruption of a strongly polarised system would be an effective control in its own right.

To say that extension of *C. nebularis* is polarised does not of course explain *why* it is polarised: that demands an explanation

of the advantages gained by a genet in establishing powerful source-sink relationships and associated channeling of resources and enhanced extension rates. These advantages may be understood both in terms of foraging theory (*cf.* Stephens & Krebs, 1986) and current concepts of ecological strategies (*cf.* Grime, 1979; Cooke & Rayner, 1984).

Foraging efficiency is dependent on patterns of resource supply and turnover. Where resources are temporarily abundant more can be gained by consuming only those most readily available before moving on, i.e. by rapid and partial turnover, than by complete assimilation. Such rapid turnover appears to be characteristic of *Cl. nebularis* which by comparison with most other common woodland litter decomposers with extensive mycelia, e.g. *Cl. flaccida , Collybia confluens, Co. dryophila, Co. peronata* and *Marasmius wynnei* Berk. & Br. has a rapid extension rate, sparse mycelium and causes relatively modest visible bleaching and breakdown of leaf litter (unpublished observations). In particular, *M. wynnei* forms extremely dense, slow-moving mycelia with a sparse but not empty centre in strongly bleached litter - the antithesis of *C. nebularis* (see Cooke & Rayner, 1984).

Exploitation of temporary abundance, in this case due to recent litter fall (*cf.* Pugh, 1980), is in turn characteristic of a ruderal life strategy which capitalises on the relative absence of environmental stress and competitors. The existence, relative to other decomposers, of such a strategy in *C. nebularis* is commensurate with its lack of combative ability in interspecific interactions.

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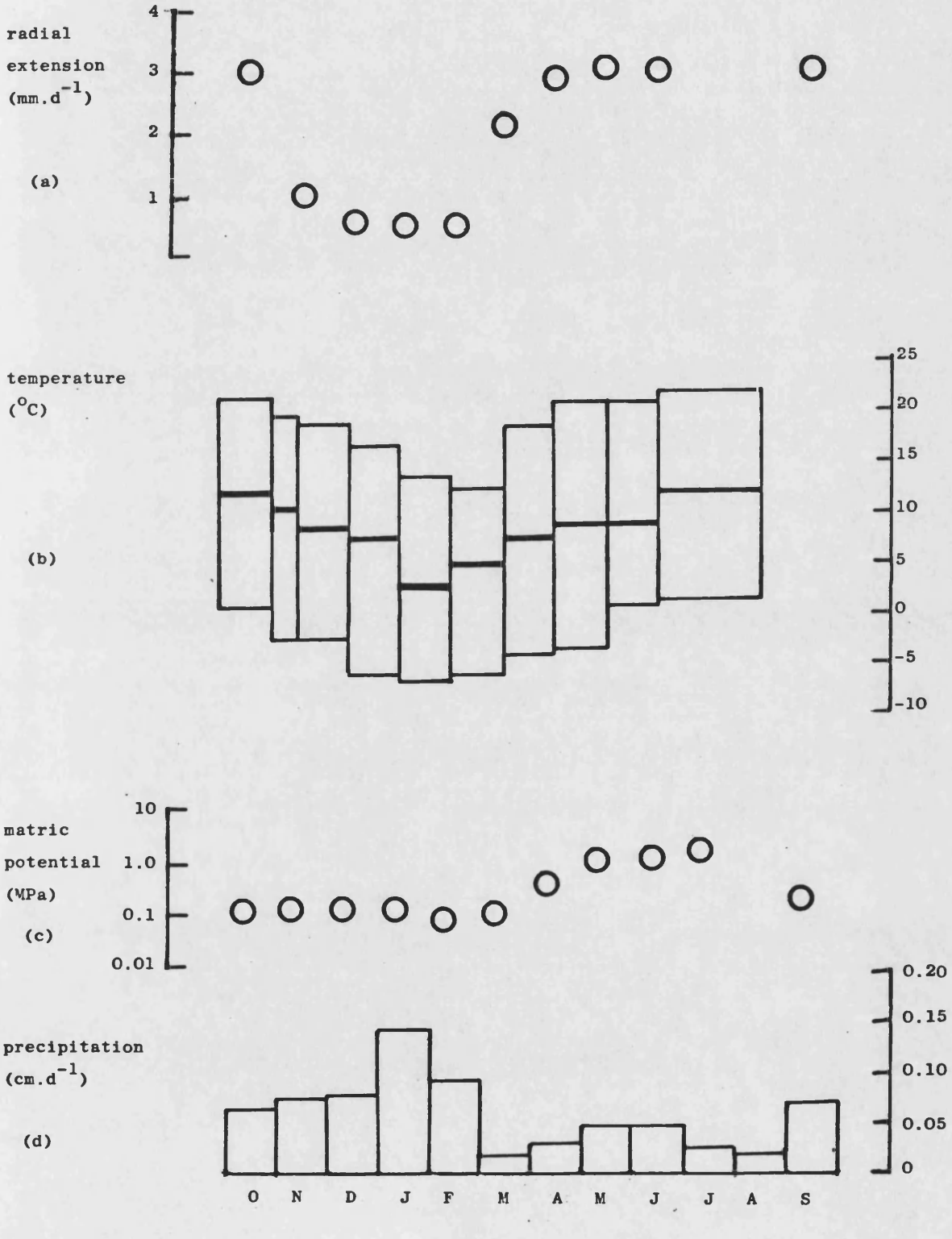
FIGURE LEGENDS

Fig. 1. (a) Mean radial extension rates of six mature fairy ring systems of *Clitocybe nebularis* from October 1983 to September 1984 at the field sites. (b) Corresponding maximum and minimum temperatures and range of exponential mean temperatures (solid bands). (c) Corresponding soil matric potentials.

Fig. 2. Mycelial cords (arrowed) at the mycelial growth front of a *Clitocybe nebularis* fairy ring system.

Fig. 3. Dense and lysing mycelium (arrowed) at the trailing edge of a *Clitocybe nebularis* fairy ring system.


radial
extension
(mm.d^{-1})
(a)
4
3
2
1
temperature
(^{o}C)
(b)
25
20
15
10
5
0
-5
-10
matric
potential
(MPa)
(c)
10
1.0
0.1
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precipitation
(cm.d^{-1})
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Figure 1.

Figure 2.

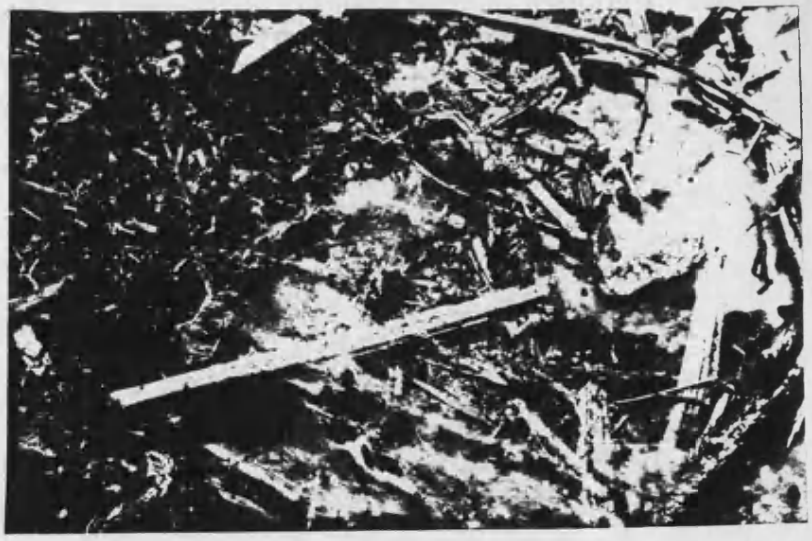

Figure 3.

CHAPTER 8.

DEVELOPMENT AND EXTENSION OF MYCELIAL CORDS IN SOIL AT DIFFERENT TEMPERATURES AND MOISTURE CONTENTS

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Cord systems of Hypholoma fasciculare, Phallus impudicus, Phanerochaete laevis, Phanerochaete velutina and Steccherinum fimbriatum developed progressively in non sterile soil from advancing mycelial fronts within which linear aggregation of hyphae was followed by lysis of diffuse mycelium.

Extension rates of the cord systems were generally linear and mostly increased with temperature from 5 to 20 or 27°. Extension rates varied between species and sometimes between strains of the same species. In S. fimbriatum an abrupt change occurred from a slowly extending, highly branched mycelium to a rapidly extending, sparsely branched cord system, which appeared to be triggered by an internal switch rather than by environmental factors.

Extension rate was little effected over the range of initial soil matric potentials from -0.007 MPa (field capacity) to -0.9 MPa, but extension was hindered above field moisture capacity and only cord systems of H.fasciculase and P. impudicus persisted. Lateral branching decreased as soil matric potential decreased.

All strains decayed wood block inocula after 70 d. The minimum temperature at which decay occurred varied between 5° to 15°; decay increased with temperature up to 20 or 27° for all species, but this increase was not always correlated with cord extension rate.

Mycelial cords are discrete filamentous hyphal aggregations formed by a variety of fungi, especially Basidiomycotina; unlike rhizomorphs, cords do not possess a defined apical growing point. They vary in complexity from simple loose associations of undifferentiated hyphae to highly organised structures containing discrete layers of hyphae serving distinct functions such as strengthening and conduction of water and solutes (Townsend, 1954; Eamus & Jennings, 1986; Watkinson, Davison & Bramah, 1981). Cords extend into nutrient deficient domain, and hence depend on resources supplied from localised depots of food bases.

Clearly, mycelial cord systems develop in a fundamentally different manner from the diffuse growth of hyphae in colonies. It is therefore of interest to know how the growth responses of cords compare to those of diffuse mycelia. Cords of dry rot fungus Serpula lacrimans, which is only found in buildings, have been studied in detail with respect to their development and growth dynamics (Butler, 1966; Watkinson, 1971; Coggins et al., 1980), and several field studies of the growth dynamics of some naturally occurring saprotrophic fungi have been made (Thompson & Rayner, 1982, 1983; Dowson, Rayner & Boddy, 1988 a,b), but investigations of the latter under controlled environmental conditions have been confined to examining the effects of light and sterile soil on extension rate (Thompson & Rayner, 1983). In this paper we report on the effects of temperature and moisture on the decay of wood block inocula, and on the development and extension of cord forming fungi in soil.

MATERIALS AND METHODS

Isolation procedure

Phallus impudicus Pers., Phanerochaete velutina (DC ex Pers.) Parmasto and Phanerochaete laevis (Fr.) Erikss. & Ryv. were isolated from cords. Freshly collected cords, 1-3 mm diam, were rinsed, cut into lengths of 3-4 cm, placed in sterile distilled water (SDW), and scraped to remove extraneous material. Cords were then transferred to universal bottles containing 10 ml SDW and agitated for 3 min on a 'Whirlymixer'. After several such washings the cords were cut into 0.5-1.0 cm lengths and placed onto malt agar (MA; 2% (w/v) Munton and Fison spray malt, 1.5% (w/v) lab M No. 2 agar) or MA plus 0.01% (w/v) novobiocin (MAN). Steccherinum fimbriatum (Pers. ex Fr.) Erikss. and Hypholoma fasciculare (Huds. ex Fr.) Kummer were isolated from wood samples collected from the field, by aseptically excising chips of wood and transferring them to MAN. All cultures were maintained at 20°.

Preparation of beech block inocula and soil

Wood blocks, (approx. 2cm x 2cm x 2cm, without bark) were cut, using a band saw, from trunks of Fagus sylvatica L. (20cm. diam), felled 1-2 weeks previously. Blocks were stored at -20° until required and then soaked in distilled water for 2 h before being autoclaved, in foil covered beakers, in batches of 20-30 for 30 min. at 121°. After cooling they were placed on 2-week-old cultures of each isolate grown on 500 ml MA in 2 l conical flasks and incubated for 5 weeks, by which time blocks were fully permeated with mycelium.

Soil tube experiments

To study cord morphology and extension rate systems were allowed to develop in tubes containing soil. Soil tubes consisted of two glass tubes: one empty tube (20 cm long and 2 cm external diameter fitted with rubber bungs at each end), was placed centrally, both longitudinally and concentrically, within a larger tube, 30 cm long and 3 cm internal diameter. The larger tube was fitted with a non-absorbent cotton wool plug at one end and 100-110g of sandy loam soil (collected from Friary Woods nr. Bath, N.G. Ref. ST 785588) was either poured or pushed evenly, avoiding compacting, into the space between the tubes until the inner tube was covered. This arrangement of tubes prevented cords from extending deep into soil, where observation would not be possible. The soil had been sieved (4mm mesh) and stored outside in a covered, drained plastic bin or allowed to air dry to known moisture contents before use.

In one experiment the soil tubes were placed upright with the plugged end in a water bath containing 10cm depth of distilled water. After 4d a continuous water gradient from field capacity (-0.007 MPa soil matric potential (SMP)) to fully saturated soil had established from the top to the bottom of the tubes. A colonized wood block, scraped clean of surface mycelium, was

then placed at the drier end above each inner tube, covered with soil and the open end of tube closed with a cotton wool plug and Cling Film (which has a high permeability to CO_2 and O_2 but reduces water loss). Tubes were then incubated in a covered water bath at 20° or 27°, in the dark in triplicate for each of the five species.

In a second experiment tubes containing soil having initial water contents of 37%, 28% and 15% (oven dry weight), which is equivalent to -0.007, -0.024 and -0.9 MPa respectively at 20°, were inoculated at one end in the same manner as just detailed, and secured at both ends by cotton wool plugs and cling film. They were then incubated horizontally at 5, 10, 15, 20, 27° in the dark, and at high humidity, which was maintained by placing trays filled with water in the base of the incubators. Each treatment was replicated three times for each of the five species. All tubes were weighed at the start and end of incubation.

A similar experimental system was used to compare cord extension rates between five different strains, in triplicate, of H. fasciculare, P.impudicus and P.velutina at an initial water content of 37% (-0.007 MPa) at 15°.

Tubes were examined at intervals of 3-4 d to record the position of the mycelial front for 70 d. Form and development of mycelia were examined and recorded by direct observation and by using an Olympus SZ-Tr stereo microscope, fibre optic illumination and OM 2N photomicrography outfit. The position of the mycelial front was marked on the outside of tubes at each examination. The recorded positions of the growth fronts were then traced on to paper from which extension rates were calculated. Decay of wood inocula was determined by comparing block density at the beginning and end of the experiment. For each species, comparisons between extent of decay at the end of the experiment under different temperature and moisture regimes were made using one-way analaysis of variance. Colours of mycelia were described following Rayner (1970).

Extension rate for each genotype, under each set of conditions was estimated by regressing distance extended against time, using the method for several y values (i.e. three in this case) for each value of x (Sokal & Rohlf, 1981) (the standard error of the slopes obtained was usually within 10% of the mean and frequently within 5% of the mean). In the experiment to determine whether different strains of the same species extended at the same rate comparisons were made between the slopes of regression of distance extended against time. In the other experiments, regressions of extension rate against temperature were fitted for tubes at each set of SMCs. Equality of slope was then tested to compare effect of temperature at different moisture contents.

Determinations of moisture content and fluxes

Soil matric potential was determined, using an adaption of the filter paper method of Fawcett & Collis-George (1967) for soil at moisture contents of 37%, 28% and 15% at 5, 10, 15, 20 or 27°. Unless otherwise stated SMP values are given for 20°.

Inoculum wood block moisture content (WMC; obtained by oven drying at 70°) was determined at the start of each experiment for a sample of blocks colonised on agar under the same conditions as those used in the soil tubes. At the end of the experiments, WMC was determined individually for the test blocks. Wood moisture content decreased in some cases (see below) and in order to estimate the flux that occurred between inoculum blocks and soil in tube systems, uncolonized, autoclaved wood blocks, of known weight and moisture content, were placed in 150ml beakers filled with soil at matric potentials of either -0.9, -0.024 or -0.007 MPa, covered with foil and incubated in triplicate at 5, 10, 15, 20 or 27° for 70 d. The blocks were weighed at various intervals and distilled water was added to the soil to maintain the initial moisture content.

Extension on agar media

Four mm plugs cut from the edge of extending colonies of H.fasciculare, P. impudicus, P.laevis, P.velutina and S.fimbriatum were placed centrally on 2% MA plates and incubated in triplicate at 15, 20 and 27° in the dark. Mean radial extension was calculated by measuring two diameters on each plate on alternate days. Resulting extension rates were compared using ANOVA.

RESULTS

Moisture relationships

Some net loss in water content occurred from the tube systems, except from those stood in water, the rate of loss being linear with time. Matric potentials decreased from an initial value of -0.9 to -1.09 and -1.2 respectively at 5°c and 27°, and from an initial value of -0.007 to -0.01 and -0.02 at 5° and 27° respectively.

From experiments involving uncolonised wood blocks in beakers of soil, it was evident that water flux also occurred between soil and uncolonized wood blocks until they reached equilibrium. Initial matric potential of uncolonised blocks was estimated as -0.035 MPa. At SMP of -0.007 MPa, WMC increased by up to 30% of initial values after 70 d. At -0.024 MPa the increase was only 5%, while at 0.09 MPa there was a 20% loss of WMC. However, the extent to which this flux would be regulated by the presence of mycelium is not known.

Morphology

In horizontal tubes. At initial SMPs of -0.9, -0.024 and -0.007 MPa and at all temperatures, mature cords of all species usually developed following hyphal aggregation within mycelial fronts and progressive lysis of hyphae and finely branched cords between the regions of greatest agregation. On reaching the end of the tubes the mycelial fronts usually became static and remained the same width, although with P.laevis there was sometimes a reduction in width

due to the continued maturation of cords from the trailing edge. Exceptions were P.velutina at 27° with -0.024 MPa and S.fimbriatum under all conditions, which occasionally produced dense highly branched mycelia without formation of mature cords. The morphology and development of cords was the same at the interface of the soil with the surface of the large tube as within soil.

The width and definition of fronts varied between species, between strains and with SMP. P.velutina generally exhibited the most diffuse mycelial fronts (Fig. 1) behind which were formed white, well defined cords, up to 1.5mm diam and branching at angles typically of 30-60° and up to 90°. Once formed, mature cords of P.velutina were very persistent, few, if any, undergoing autolysis. At -0.9 MPa, tips of P.velutina became discoloured from white to cinnamon after extension ceased.

P.laevis formed a distinct, sinuous, loosely aggregated, highly branched front (Fig. 2). Its cords were much less persistent, and many lysed leaving only a few major cords which were up to 2mm diam (Fig. 3). However, under a regime of 27°, -0.024 MPa, and under all temperature regimes with -0.007 MPa, all minor and major cords lysed once extension had ceased at the end of the tube.

At all temperatures, P.impudicus tended to produce a distinct fan at its advancing front at -0.024 MPa (Fig. 4), a less distinct fan at -0.9 MPa and very variable fronts at -0.007 MPa. Cords were white, persistent, well defined, and up to 1.5mm diam, although they were variable, sometimes being less than 1mm diam. Branch angle, up to 90°, and frequency were also variable.

H.fasciculare produced the most distinct front composed of white/buff acutely branched mycelial fans from which ochreous/cinnamon cords (Fig.5) developed between areas of lysis, and after progressive pigmentation of aggregated regions (Fig. 6-8). The cords were up to 1.5mm diam. and possessed little tensile strength. Few branches remained on mature cords. Mycelium restricted from extending by compacted soil became highly branched and powdery in appearance. There was a strikingly greater development of lateral

branches in cord systems growing through soil at -0.9 and -0.024 MPa as compared with -0.007 MPa.

Fronts of S.fimbriatum were palmate (Fig. 9) and gave rise to initially white/rosy buff cords which became speckled with sepia on ageing (Fig. 10), especially as soil moisture increased. They were up to 1.5mm diam and possessed a high tensile strength. Occasionally the loosely interwoven tips branched dichotomously around soil crumbs. A dense mycelium with short internodes and wide branch angle often developed and extended at about half the rate of acutely branched well-defined cords under the same conditions (Fig. 11). After 25 to 35 d incubation the leading edge of this dense mycelium usually changed abruptly, via an increase in internode length and decrease in branch angle, to give rise to normal cords. This switch occurred in all temperature and moisture combinations but one (27°, -0.024 MPa). A similar morphogenetic pattern occurred with H.fasciculare but only at -0.024 MPa (Fig. 12).

Cord tips of H.fasciculare, P.velutina and P.laevis, but not S.fimbriatum and P.impudicus, became increasingly compact with increasing temperature (Fig. 13-15). Tip regions of H.fasciculare were diffuse in moisture gradient tubes and at -0.007 MPa, but compacted at -0.024 and -0.9 MPa. At -0.024 and -0.007 MPa, fully formed cords of S.fimbriatum and P.velutina occasionally de-differentiated at a point on their surface to produce a highly branched mycelium which extended up to 3 mm either side of the original cord to cover adjacent crumbs of soil (Fig. 16).

Extension rate

The extension rate of cords in horizontal tubes was usually linear at all SMP's, and the variation was low, the error for triplicates was always less than 10% of the mean value. However, there was often a significant difference ($P \leq 0.05$) in extension rate both between species and between strains of the same species. At 15°, -0.007 MPa, means for different strains ranged from 0.0-3.8 $mm.d^{-1}$ for P.velutina, 1.4-2.4 $mm.d^{-1}$ for P.impudicus and 1.0-1.8 $mm.d^{-1}$ for H.fasciculare. Strains used in the other experiments had extension rates close to the means of each species.

Minimum conditions for growth varied with species, only P.velutina (Fig. 17) and H.fasciculare (Fig. 18) had significant ($P \leqslant 0.05$) extension rates (0.9 and 1.2 $mm.d^{-1}$ respectively) at 5° at -0.024 and -0.007 MPa. Significant ($P \leqslant 0.05$) extension of S.fimbriatum (Fig. 19; 1.0 $mm.d^{-1}$) and P.laevis (Fig.21; 2.0 $mm.d^{-1}$) occurred at 10°, -0.024 MPa, whilst 15° (-0.9, -0.024 and -0.007 MPa) was the lowest temperature at which P.impudicus (Fig. 20) showed any noticeable extension (1.3 $mm.d^{-1}$).

Extension rates usually increased with temperature, up to 20° for P. velutina, S.fimbriatum and P.impudicus, and 27° for P.laevis and H.fasciculare (Fig. 17-21). The conditions for maximum extension and the rates attained for each species were 8.0, 7.0, 4.7, 3.3 and 3.2 $mm.d^{-1}$ for P.velutina (at -0.024 MPa; 20°), P.laevis (-0.024 and -0.007 MPa; 27°), S.fimbriatum (-0.024 MPa; 20°), P.impudicus (-0.9 MPa; 20°) and H.fasciculare (-0.007 MPa; 27°) respectively. SMP did not usually have a significant ($P \leqslant 0.05$) effect on extension at 5-15°, but did so at higher temperatures.

Extension of cords along continuous moisture gradients in vertical tubes was usually linear to a point where soil became saturated (greater than field capacity). Here extension was inhibited or ceased altogether and cords of P.laevis, P.velutina and S.fimbriatum lysed. Generally, extension rates were lower than those observed at -0.9, -0.024 or -0.007 MPa (Fig. 17-21), but one exception was P.velutina which extended at 1.5 $mm.d^{-1}$ at -0.024MPa, 27°, which was significantly ($P \leqslant 0.05$) slower than growth in moisture gradient tubes at 27° (1.8 $mm.d^{-1}$). This was associated with the fact that under the former conditions P.velutina extended as mycelium rather than as cords.

Extension rates of mycelia on agar (Table 1) followed a similar trend to the extension of cords in soil tubes with P.velutina > P.laevis > S.fimbriatum > H.fasciculare > P.impudicus. However, under optimal conditions, extension rates of cords in soil tubes were more than two-fold greater than for mycelia on agar for species other than H.fasciculare.

Decay of wood blocks

All species caused significant ($P \leq 0.05$) decay of wood block inocula after 70 d incubation under optimal conditions. The weight loss generally increased from 5 to 27° (Fig. 22-26), although the relationship differed from that seen for extension (Fig. 17-21).

At 5° only P.velutina caused a significant ($P \leq 0.05$) dry weight loss (5%), whilst P.impudicus only caused significant decay at and above 15° (up to 10% weight loss). P.velutina and H.fasciculare (Fig. 22, 23) both brought about significantly ($P \leq 0.05$) greater decay, at all temperatures and moistures, than any of the other three species. Maximum decay by P.velutina and H. fasciculare occurred in the moisture gradient tubes.

At ecologically significant temperatures (5-20°) decay by P.velutina and S.fimbriatum was little affected by soil matric potential. Conversely, decay by H.fasciculare and P.laevis (Figs. 23, 26) was rather more affected by SMP than was their extension (Figs. 18, 21).

Decay was not directly correlated with rates of extension, especially in P.velutina which at -0.007 MPa brought about similar amounts of decay (43% and 37%) at 20° and 27° respectively but exhibited vastly different rates of extension (8.0 and 1.5 $mm.d^{-1}$), cords being produced at the lower temperature and a slow, densely growing mycelium at the higher temperature. A similar situation occurred with S.fimbriatum. In P.laevis, a ten-fold difference in decay (2.5 - 25% dry weight loss) occurred between -0.024 and -0.007 MPa at 27°.

Although decay of inoculum blocks, like extension, usually increased with temperature up to either 20 or 27° the degree to which soil moisture affected the ratio between the rate of decay and extension varied between species. For example, at 15° P.velutina caused greater decay per unit extension at -0.007 MPa than at -0.024 or -0.9 MPa (Fig. 17, 22), whereas H.fasciculare caused greater decay per unit extension at -0.024 MPa than at either -0.9 or -0.007 MPa (Figs. 18, 23).

DISCUSSION

As with mycelia growing on agar plates there was generally a linear relationship between temperature and extension rate of cords in soil. However, extension rates achieved under optimal conditions were up to several fold higher than that of diffuse mycelia on 2% malt agar (Thompson & Rayner, 1982, 1983; Boddy, 1983), suggesting some channeling of growth resources within the cord systems. Although decay rates of wood blocks, like extension rate, increased up to an optimum temperature, the relationsip between temperature and decay differed qualitatively from that with extension. This difference was especially noticeable in Phanerochaete velutina where maximum decay at 27° was similar to that at 20° but extension rate at 27° only 20% of that at 20°. These findings suggest that extension of cords and decay represent distinctive modes of functioning in the mycelium which may be both metabolically competitive and involve metabolic pathways which are differentially affected by environmental conditions (Gregory, 1984; Sharland, Burton & Rayner, 1986; Rayner, Boddy & Dowson, 1987). With respect to the latter point, an interesting observation has been reported for Phellinus tremulae (Bond.) Bond. & Borisov, which causes heartrot of aspen (Niemela, 1977). This involved a mycelial dimorphism between an appressed pigment-producing colony form producing extracellular laccase and tyrosinase, and an aerial

form with peroxidase activity. The appressed form had a distinctly higher maximum temperature for extension growth than the aerial form.

Evidence for a degree of competition between exploratory outgrowth and decay has been provided by Thompson & Rayner (1982, 1983) who found that colonized wood blocks inoculated into tubes of non-sterile soil decayed less than blocks inoculated into sterile soil, where outgrowth was more diffuse and extended less rapidly. Further evidence for a reciprocal relationship between decay and outgrowth has come from recent studies in which uninoculated 'bait' wood blocks or other organic substrata were placed into trays of soil some distance away from wood block inocula pre-colonized by H.fasciculare or P.velutina (Dowson, Rayner & Boddy, 1986; unpublished). Decay of the inoculum wood block was less in trays provided with additional wood baits than in controls to which no bait had been added.

Soil matric potential had variable effects on extension, decay and morphogenetic patterns. At temperatures of less than 20°, and for some species at 25°, extension rates of cords were hardly affected by initial soil matric potentials of -0.007 MPa (field capacity), -0.024 and -0.9 MPa. For Phanerochaete velutina this contrasts markedly with the affect of water potential of agar media on mycelial extension (Boddy, 1983), where a positive relationship was found between water potential and extension rate; extension was reduced from a maximum value of 4.7 $mm.d^{-1}$ to less than 3 $mm.d^{-1}$ at -0.9 MPa, and extension ceased just below -3.0 MPa. This may be due partly to the fact that the salts used to control agar solute potential may have been slightly inhibitory, but also to the fact that in the soil tubes water is probably supplied to the growing mycelial front from the wood block inoculum. Eamus and Jennings (1986) reported that significant growth occurred in cords of P.impudicus whenever a positive turgor pressiure was present, and that increase in turgor pressure resulted in increase in extension rate. Since extension rates were similar in soil tubes between -0.007 MPa and -0.9 MPa it seems likely that either turgor pressure generated within the inoculum blocks was sufficient to prevent limitation of extension rate, or that wood block matric potential

was the same irrespective of soil matric potential. The latter was probably true at the start of the experiment although, as indicated by the water flux experiments, wood block matric potential would be expected to change during the experiment. An additional complication results from the fact that metabolic activity generates water (complete degradation of 1 g of cellulose provides 0.555 g water, Griffin, 1977).

Limitation of extension in the moisture gradient tubes may be attributable to poor aeration, and here it was interesting to note that P.impudicus the species with the most apically dominant cords, was also the most tolerant of these conditions. The true rhizomorphs produced by Armillaria spp. are usually regarded as being able to tolerate anoxic conditions via diffusion of oxygen through a central air channel (Smith & Griffin, 1971).

The cause of the increased apical coherence of cords produced at lower SMCs is not certain but may relate to the strength and/or diffusibility of recruitment signals regulating apical dominance (Rayner & Franks, 1987). Interestingly, some species, e.g. Coriolus versicolor and Stereum gausapatum, which do not normally produce mycelial cords, sometimes form aggregated structures at low water potentials on agar media (Boddy, Gibbon & Grundy, 1985; L. Boddy, unpublished).

The range of outgrowth patterns and decay rates exhibited by the diffe rent fungi in the present study can be understood in terms of the spectrum of differing colonization strategies that they have adopted in response to environmental stress, competition and the spatiotemporal distribution of woody resources in soil and litter (Rayner & Franks, 1987). As a working hypothesis, outgrowth on a broad mycelial front with considerable commitment of biomass, rapid decay rates and a dynamic mycelial domain may have evolved in response to a high probability of encountering resource units suitable for colonization close to the food base. In undisturbed habitat this probability would be greater in fungi which are non-selective in their requirements for particular resource types or microenvironmental regimes, associated with the ability to replace

resident organisms. By contrast, channeling of biomass into more rhizomorphic outgrowth would be advantageous to species with a low probability of encountering suitable resource depots, due to intolerance of certain micro-environmental factors, selectivity for a particular type of resource, or inability to compete with other species and hence colonize all but 'virgin' resources.

Putting these observations, and those from other studies, (Dowson, Rayner & Boddy, 1986; 1988a,b,c) into a broader ecological context, H.fasciculare and P.velutina are seen as non-selective and aggressively combative organisms with dynamic mycelial domain, and result in rapid decay rates. P.laevis, although aggressively combative appears to be more selective for resource type and micro-environmental regimes; British strains of this fungus, associated with Fagus sylvatica litter, are intolerant of high moisture and have a high optimal temperature for growth and decay. These features may reflect its sparse distribution. Steccherinum fimbriatum combines aggressive and defensive combative strategies and non-selectivity for resource types, whilst appearing to be particularly adapted to growth in soils subject to widely fluctating moisture contents. The thick rubbery mycelial mat that it forms over the outside of colonized wood may protect the internal mycelium from water flux. Such adaption may underlie its ability to switch spontaneously from slow-dense to fast-effuse mycelial outgrowth patterns. This abrupt alteration in morphogenesis corresponds to similar transitions in diffuse mycelia, which are sometimes prompted by growth at low water potential (Coggins et al., 1980; Rayner et al., 1985; Boddy, 1983; Rayner & Coates, 1987). P.impudicus produces a persistent mycelial cord network, parts of which supply its basidiocarps, associated with a relative lack of aggressive combative ability, slow decay rate and rhizomorphic outgrowth patterns.

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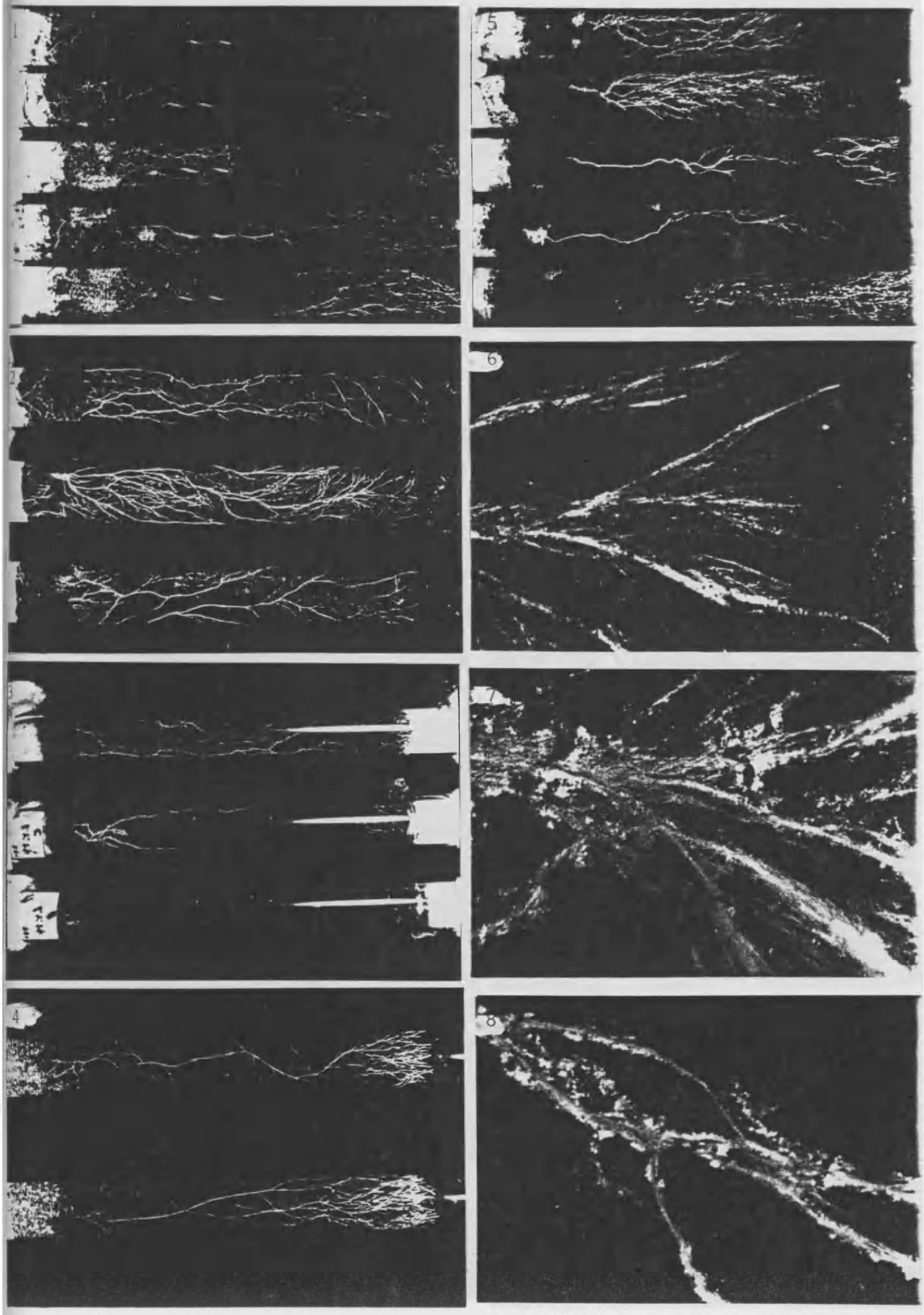
Figures 1-8). Morphology of P.velutina, P.laevis, P.impudicus and H.fasciculare. 1) Variation among five isolates of P.velutina at 15° with -0.007 MPa. 2) Cords of P.laevis at 20° with -0.9 MPa. 3) Cords of P.laevis following lysis of lateral branches at 20° with -0.02 MPa. 4) P. impudicus at 20° with -0.02 MPa. 5) Variation among five isolates of H.fasciculare at 15° with -0.007 MPa. (6-8) Developmental sequence of major cord formation in H.fasciculare; 6, leading edge; 7, midway along tube; 8, mature cord near to inoculum block.

Figure 9-16). Morphology of S.fimbriatum and H.fasciculare. 9) Palmate fronts (→) of S.fimbriatum at 20° with -0.9 MPa. 10) Sepia coloured patches (→) on mature cords of S.fimbriatum at 20° with -0.007 MPa. 11) Slow dense (s.d.) mycelium of S.fimbriatum and fast extending (f.e.) cords at 20° with -0.007 MPa (compare low frequency of lateral branching with higher frequency in 2a). 12) Transition from s.d. to f.e . in H.fasciculare at 20° with -0.02 MPa. 13-16) Increasing hyphal aggregation with increasing temperature in the leading edge of H.fasciculare, at -0.007 MPa: 13, 15°; 14, 20°; 15, 27°. 16) The differentiation of cords of S.fimbriatum to a highly branched mycelium (→).

Figures 17-21 Linear extension rates of cords along soil tubes at different temperatures and soil moisture contents. 17) P.velutina; 18) H.fasciculare; 19) S.fimbriatum ; 20) P.impudicus; 21) P.laevis, at initial soil matric potential -0.9 MPa (oven dry wt.) (-■-), -0.024 MPa (-□-), and -0.007 MPa (-○-), continuous moisture gradient 30-50% (-●-). Error bars are omitted for clarity. All points for extension, when not touching, were significantly different ($P < 0.05$).

Figures 22-26 Decay of 8cm^3 beech inoculum blocks in soil by 22) P.velutina; 23) H.fasciculare; 24) S.fimbriatum; 25) P.impudicus; 26) P.laevis. At initial soil moisture content -0.9 MPa (oven dry wt.) (-■-), -0.02 MPa (-□-), -0.007 MPa (-○-), continuous moisture gradient 30-50% (-●-). Error bars are omitted for clarity. All points for decay, when not touching, were significantly different ($P < 0.05$).

Table 1. Extension rates of P.velutina(Pv); H.fasciculare(Hf); S.fimbriatum(Sf); P.impudicus(Pi) and P.laevis (Pl) (mm.d^{-1}) on 2% malt extract agar at 15, 20 and 27°, with 95% confidence intervals.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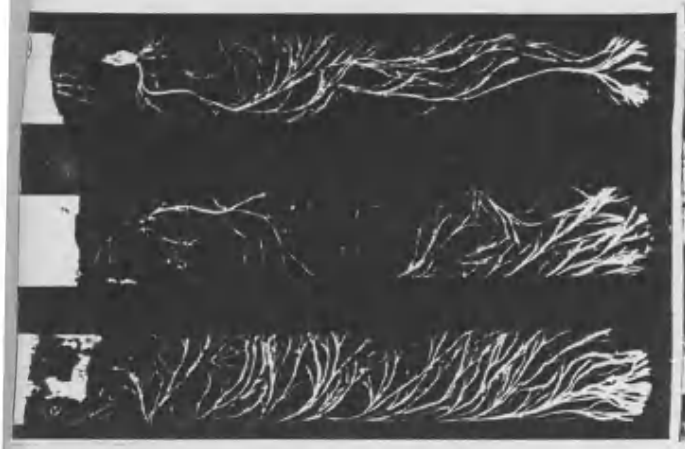
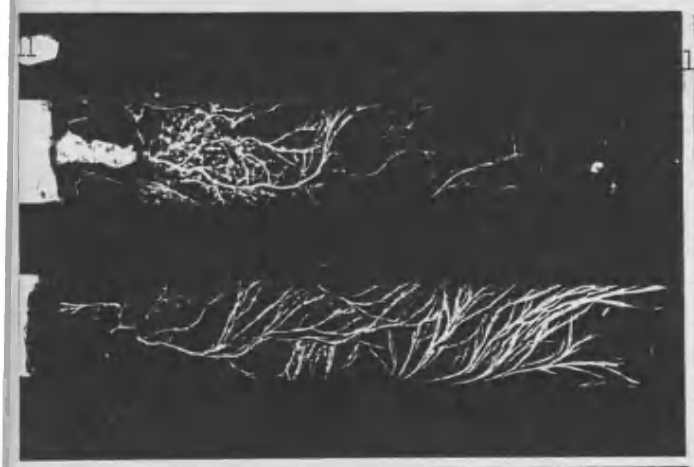
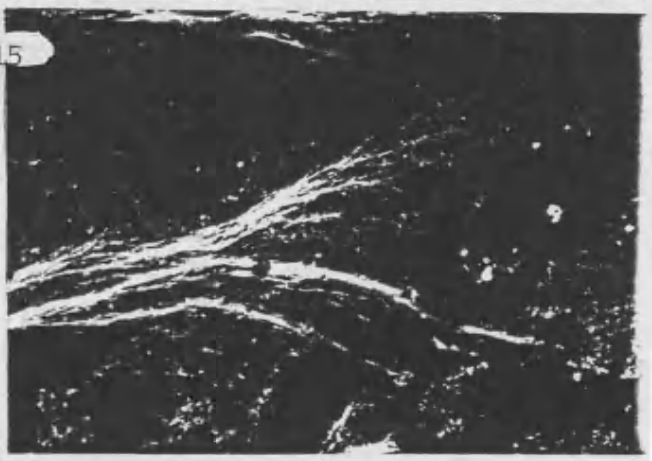
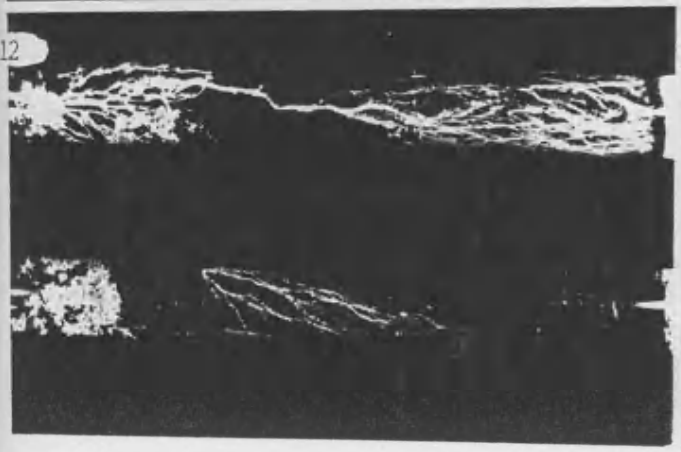
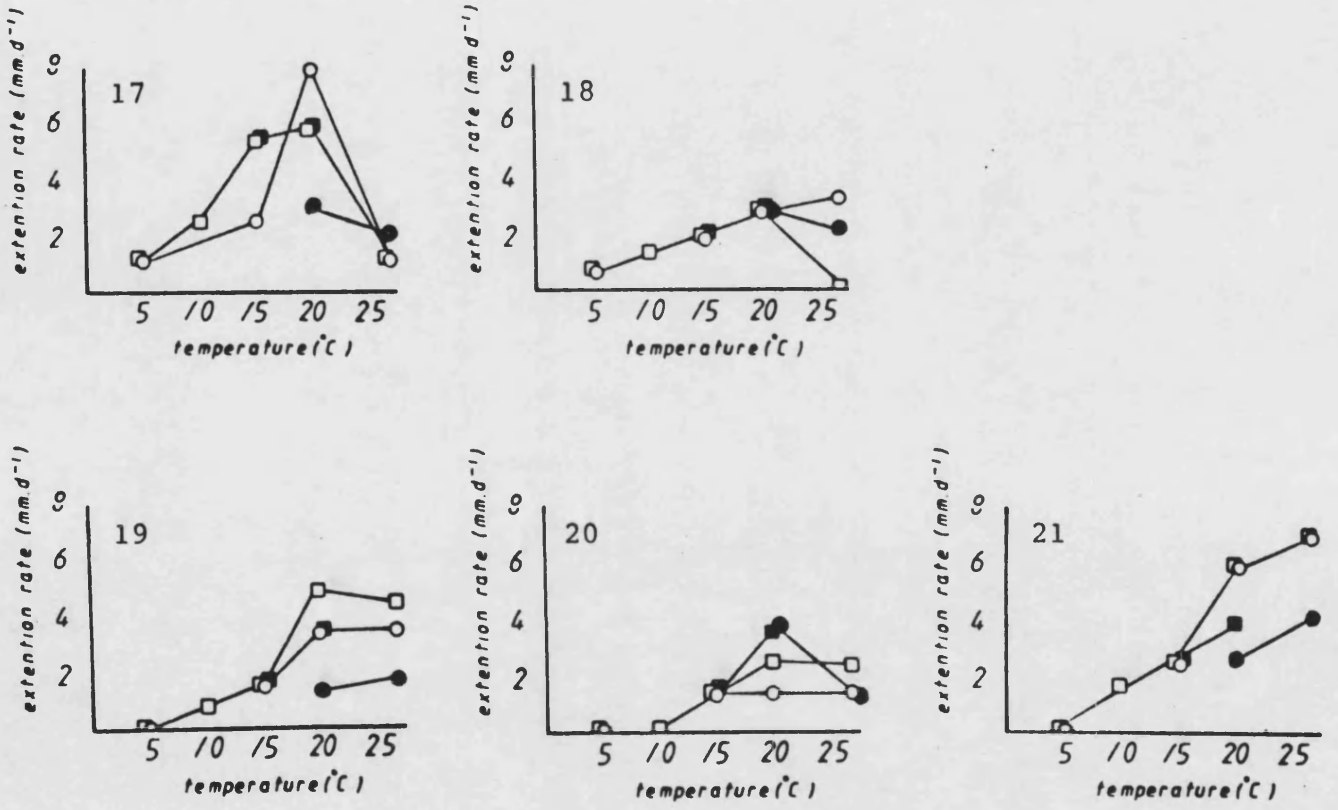


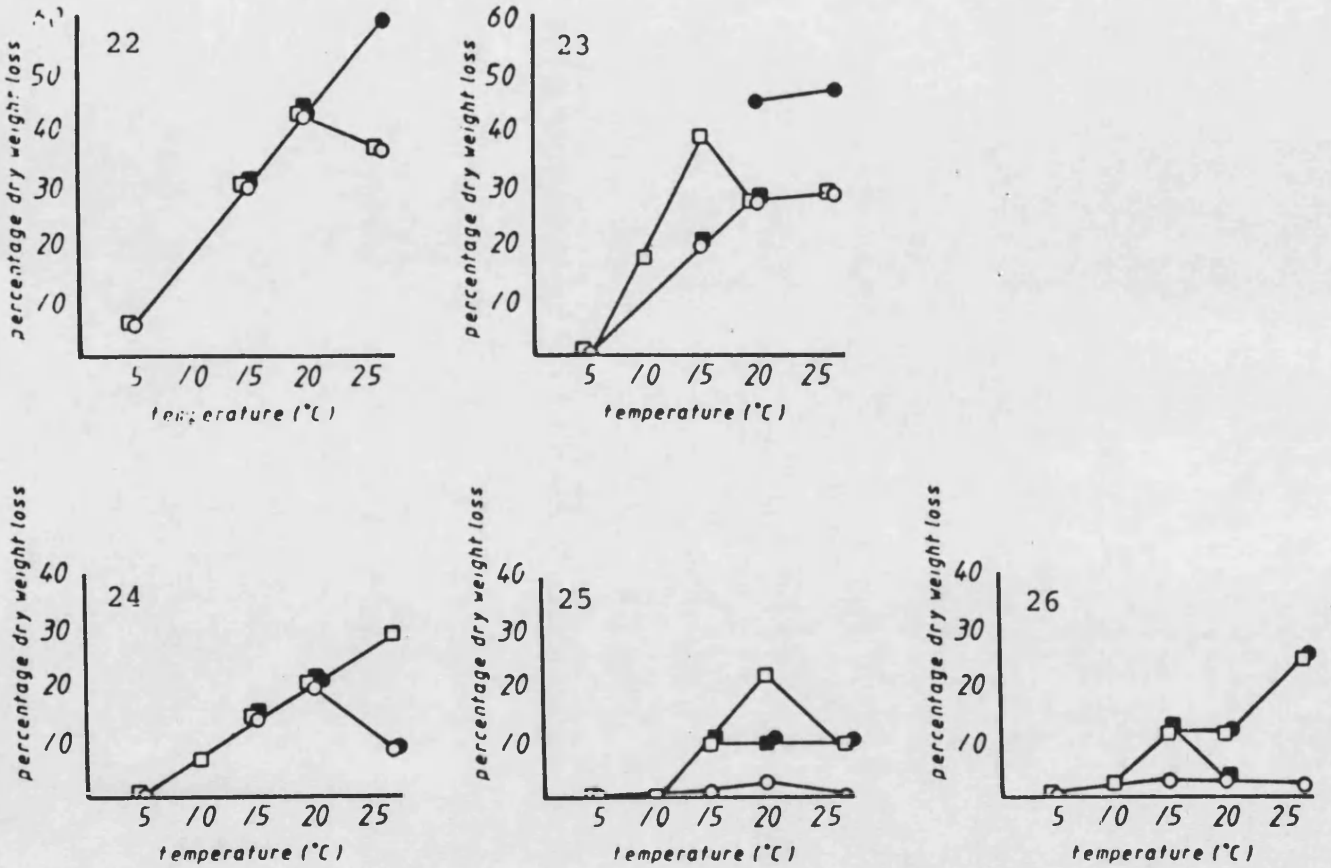








Figures 17-21.



Figures 22-26.

CHAPTER 9.

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GENETIC INTERACTIONS AND DEVELOPMENTAL VERSATILITY DURING ESTABLISHMENT OF DECOMPOSER BASIDIOMYCETES IN WOOD AND TREE LITTER

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INTRODUCTION

Crucial as they are in community biology, the processes by which decomposer basidiomycetes (i.e. excluding biotrophs such as the Ustilaginales and Uredinales) establish themselves within natural habitats have been neglected. This neglect has probably been partly due to the naive view of establishment as simply involving the arrival of propagules at or below the resource surface, followed, under suitable conditions, by mycelial outgrowth. However, the issues are obviously far more complex because establishment processes necessarily involve a dynamic interplay between the activities of the basidiomycete thallus and both abiotic and biotic components of its microenvironment, the biotic components including other genotypes of the same species. In consequence, the thallus is exposed, often sequentially, to selection pressures favouring different and even opposite attributes. For example, attributes favouring rapid ramification in a domain may not be appropriate to defence or effective exploitation of resources in that domain.

An impoverished view of the nature and properties of basidiomycete mycelium, as something resembling animated cotton wool (Wood, 1985), has further clouded understanding of the manner in which these changing selection pressures can be coped with. For example, it was once widely considered that sufficient genetic flexibility could be achieved by hyphal fusion (anastomosis) between different mycelia of the same species to form a complex, physiologically unified genetic mosaic. From this mosaic, genetic components best fitted to the changing ecological settings faced by the mycelium as

colonization proceeded could be selected sequentially (Burnett, 1965). Such a pattern of behaviour would be unique amongst eukaryotes and at variance with modern ideas about the action of natural selection on individuals rather than groups (Williams, 1971; Carlile, in press).

Growing awareness of two fundamental attributes places these issues in a different perspective, suggesting that the mycelium of basidiomycetes is a body form which resembles that of fully-fledged multicellular organisms. First, being composed of an intercommunicating system of apically extending, branching tubes (hyphae) filled with protoplasm, the mycelium is like an indeterminate embryo, with different parts able to select from a range of alternative modes of development to suit distinctive functional requirements (Rayner & Coates, in press). Thus a series of superimposable switch mechanisms control the outcome of contrasting patterns of morphogenesis such as cells and hyphae, branching and extension, diffuse and coherent growth, and juvenility and senescence. It is this facility, rather than genetic heterogeneity, which confers on the individual the developmental versatility enabling it to span the heterogeneous, changing and often discontinuous niches it occupies naturally.

The second important attribute is that basidiomycete hyphae possess powerful recognition responses which condition the occurrence and outcome of fusion with other hyphae, or propagules, of the same or different genotype (Rayner, 1986*a*). A long-range signalling system determines the primary ability of hyphae to grow towards receptive sites prior to fusion with other hyphae, conidia or basidiospores, and generally seems to operate within a species, or between closely related species, regardless of genotype. Fusion and subsequent reactions are determined by contact stimuli, which do depend on genotype.

Self-fusions, within the same thallus, or between thalli of identical genotype, occur readily and convert the mycelium from a radiate communication system to a network. Protoplasmic destruction and erosion of septa associated with nuclear migration are not found, although in strictly monokaryotic and dikaryotic mycelia a remarkable process of nuclear replacement occurs in recipient compartments (Aylmore & Todd, 1984; Todd & Aylmore, 1985; Ainsworth & Rayner, 1986).

Non-self interactions between thalli differing in genotype at typically multiallelic or polygenic recognition loci can be of three distinctive types. Sexual acceptance allows entry of donor nuclei into

recipient hyphae, usually followed by nuclear migration associated with erosion of septa. It is conditioned by the presence of complementary mating alleles between interacting homokaryons, or homokaryons and heterokaryons. Rejection responses, associated with somatic incompatibility which obviates formation of unified mosaics (see above), involve what appears to be a programmed cycle of protoplasmic vacuolation and destruction in contiguous compartments. These responses occur between homokaryons which are not mating-competent and generally in interactions involving heterokaryons, where their presence is related to the non-receptiveness of the mycelia formed by mating to nuclear migration. Parasitic interactions are often initiated by similar signalling systems, but they do not usually result in protoplasmic continuity: entwining and penetration of recipient ('host') hyphae are the usual outcome and rejection responses are not elicited – at least initially.

The importance of these two major attributes of basidiomycete mycelia in determining patterns of establishment in wood and litter will now be examined. First, the nature of the problems which successful colonists must solve before establishment in these habitats will be considered, followed by an account of the wide variety of colonization strategies used to solve these problems. Developmental versatility and recognition responses can then be understood within the context of the distinctive requirements of different colonization strategies. It should be noted that whilst this discussion is mostly limited to basidiomycetes, many of the arguments will apply to other fungal groups, especially ascomycetes. Nonetheless, those basidiomycetes which degrade refractory lignocellulosic substrates, operate over years, decades or even centuries and this underlies the diversity of their colonization strategies and their developmental versatility, making them an excellent example for discussion.

PROBLEMS OF ESTABLISHMENT IN WOOD AND LITTER

For the purposes of this chapter, 'wood' will be taken to include all the major durable components, ≥ 1 cm diameter which are not shed from trees on a regular seasonal or annual basis. Litter includes all the fallen debris, excluding wood, which tends to accumulate at ground level below trees. As such, wood and litter provide exceedingly heterogeneous habitats and a wide variety of problems for potential basidiomycete colonists (Fig. 1). Accordingly, four principal determinants of patterns of establishment can be identified: the

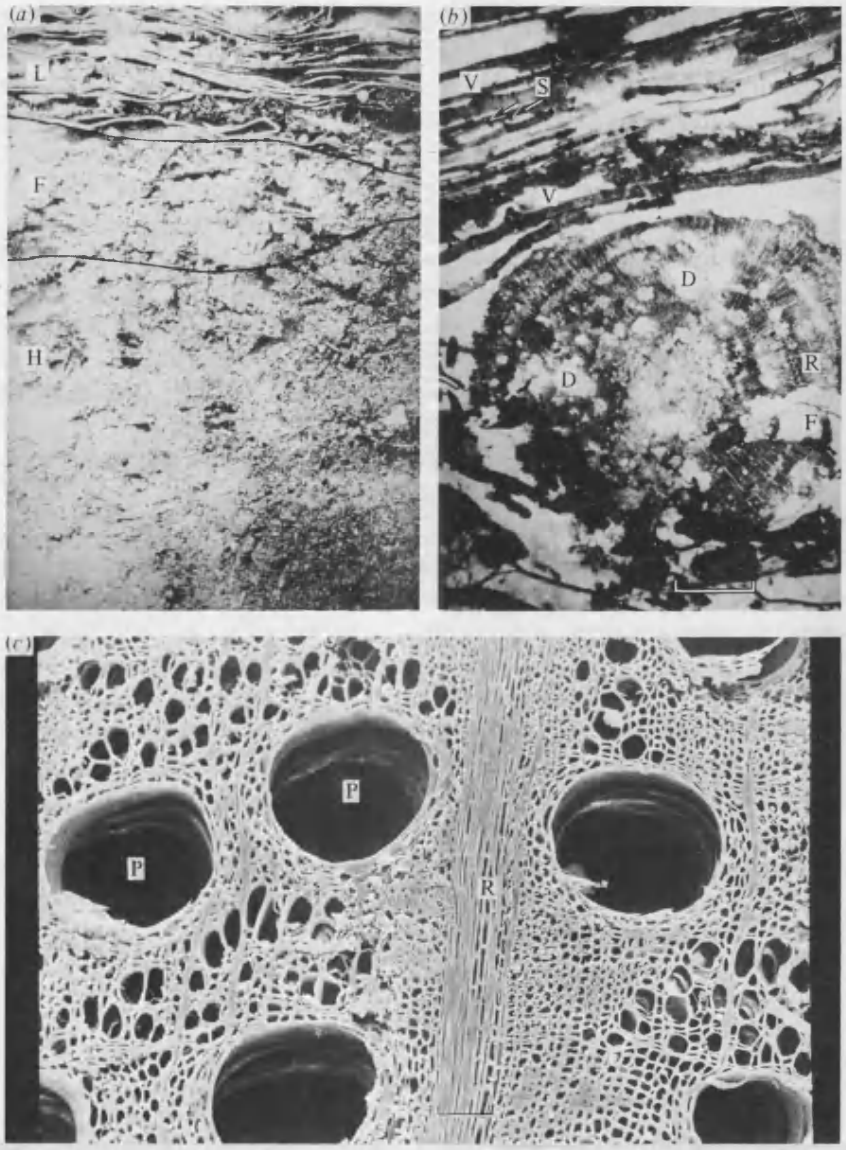


Fig. 1. Spatial heterogeneity of wood and litter microhabitats. (*a*) Detail of a gelatine-embedded vertical section through the top 5 cm of a well developed organic soil in a *Castanea sativa* (sweet chestnut) woodland showing the litter (L), fermentation (F) and humus (H) sub-horizons. (From Swift, Heal & Anderson, 1979). (*b*) Detail of a section through the litter layer and a rotten twig in the same site as (a), illustrating voids between leaves (V), voids resulting from decomposition (D), anatomical voids of the vessels and medullary rays (R), fungal hyphae (F) and dark fungal stroma (S). Scale bar = 1 cm. (From Swift, Heal & Anderson, 1979.) (*c*) Scanning electron micrograph of *Quercus* sapwood showing different sized vessels (P) and medullary ray cells (R). Scale bar = 100 μm. (Courtesy of M. Hale, unpublished.)

spatiotemporal distribution of resources, the quality of these resources, microclimatic conditions, and the extent to which a resident microflora has already become established.

Discontinuous versus continuous distribution of resources

As is implicit in the above definitions, critical differences occur between wood and litter with respect to the times and places at which they become available for colonization by basidiomycetes. These differences may be the primary reason for the incomplete, but nevertheless clear division of *K*-selected (i.e. vegetatively persistent – see below) decomposer basidiomycetes into wood- or litter-inhabiting classes.

With respect to wood, colonization is often – perhaps almost invariably in undisturbed woodlands – initiated in the standing tree (Rayner & Boddy, in press). Understanding of the processes of colonization by wood decomposers must therefore begin with consideration of factors governing establishment before fall. Furthermore, both before and after fall, woody resources are distributed discontinuously, so that fungal colonists must possess effective means of migration.

With respect to litter components, their duration on the standing tree is so much less than that of wood that there is little opportunity for establishment of basidiomycetes prior to fall except for a few highly specialized forms and true parasites. Hence the major opportunity for decomposer basidiomycetes to colonize occurs at ground level, where a second fundamental difference between wood and other litter becomes apparent. Whilst individual components of the litter, e.g. individual twigs, petioles and fruits, are discontinuously distributed, in the mass these components form a relatively homogeneous layer which provides a distinctive habitat. A third major difference is that this layer is regularly or continuously replenished by seasonal or continuous litter fall. Seasonal litter fall in particular will alter microenvironmental conditions radically as well as enriching the habitat with new substrates for exploitation by the decomposer community.

Resource quality and microclimate

The microenvironment of basidiomycete thalli growing in wood and litter is determined by the interaction between *resource quality*, that

is intrinsic physicochemical properties such as structure and chemical composition, and *microclimate*, which encompasses extrinsic conditions, notably of moisture, temperature and aeration. A detailed discussion of the microenvironments occurring within wood and litter systems has been provided by Boddy (1984). Here the most salient features determining the direction and extent of basidiomycete growth will be discussed in outline. These are considered to be first the presence of constitutive or induced barriers limiting access to nutrient resources within the plant tissues, secondly the occurrence of unfavourable moisture and gaseous regimes, and thirdly the presence of allelopathic substances.

Wood

In wood, resource quality and microclimate are influenced by the primary nature of this tissue as a device for carrying water and minerals from an underground source through the aerial environment to a distant photosynthesizing canopy. Trees have evolved a structure which provides for conduction of water in long columns under considerable tension whilst conserving this commodity by limitation of access of gases to the functioning conduits through cavitation or evaporation. The resulting plumbing systems, combined with the mechanisms for their repair and maintenance, coincidentally create a spatiotemporally highly heterogeneous set of microenvironmental conditions for establishment of fungal growth (Rayner, 1986*b*).

The predominantly axially oriented systems of conduits, together with the life support system of radially aligned medullary rays (Fig. 1c), provide potential routes of access for fungal hyphae, which in the absence of other constraints will result in wedge-shaped, columnar colonization zones. However, in the living tree, the presence of an intact bark layer impedes access to these passageways. Moreover, functionally intact sapwood filled with water will be inimical to mycelial growth, although access of air or gas to these passageways, resulting from injury or stress to the tree, cavitation or heartwood formation, will counteract this. However, alleviation of one problem for the fungus commonly introduces others. These include the formation of sealant zones (by suberization and production of gums, resins or tyloses) and infusion of the tissues with allelopathic chemicals which are absent from functionally intact sapwood. Furthermore, although the gaseous phase is often prominent in older wood, it is often relatively anoxic and enriched with carbon dioxide (Rayner, 1986*b*).

In dead wood, barriers to fungal growth will invariably be constitutive rather than induced. Microenvironmental conditions of moisture and temperature will fluctuate in response to the external environment whilst gaseous regimes will be related to both external conditions and the activity of the decomposer community. Furthermore, the bark which formerly had been a barrier may now shelter the wood from external extremes and provide readily available nutrients from the cambial layer.

Litter

Microenvironmental conditions in litter need to be considered at two levels – within the litter system as a whole, and within individual litter components. With respect to the latter, distinctive conditions result from the presence of an array of allelopathic chemicals and anatomical features. However, the relatively small size of litter components and their greater surface area to volume ratios allows them to equilibrate more readily with ambient conditions. Induced barriers are of lesser importance, given that basidiomycete colonization occurs after fall.

Regarding litter systems as a whole, microenvironmental conditions and routes of access for fungi will, as in wood, be governed by systems of fluid-filled voids, and there will be marked stratification below the surface layer (Fig. 1*a*, *b*). However, by comparison with wood, the void systems will be more heterogeneous and discontinuously distributed. Also the relative permeability of the outer layer will allow much more rapid gaseous exchange with the external atmosphere. Hence, gaseous composition will often, except in waterlogged situations, approximate to atmospheric air. Diffusion paths will be short and the desiccating or fluctuating moisture conditions in surface layers will give way to a more constant environment further down. Finally, the mobility of macro- and microfauna within the litter system provides a further point of departure from wood, at least during early stages of decomposition (see Anderson, this volume).

Extent of prior colonization

Of obvious significance to a colonist is whether or not, when it arrives, its potential habitat is already occupied, and if it is, by what. If the habitat is occupied, then successful establishment may depend on mechanisms allowing replacement of the previous resident(s).

If the habitat is unoccupied, then success may be determined by the ability to exploit this situation as rapidly as possible, particularly by utilization of easily assimilable substrates before only the more refractory lignocellulosic ones remain. Unless deliberately cut or broken off by wind or storm, wood will generally be substantially decayed by the time it becomes 'available' to non-pathogenic decomposer basidiomycetes at ground level. By contrast, litter, although colonized by phylloplane organisms, pathogens and endophytes, will not contain a firmly established community of decomposer basidiomycetes at the time of fall.

COLONIZATION STRATEGIES

The foregoing account has summarized the widely varying biotic and abiotic environmental factors which limit the establishment of decomposer basidiomycetes in wood or litter. In order to understand how these lead to diverse modes of establishment it is necessary to identify the behavioural attributes required to overcome the various constraints. This can be done by considering first the general ecological strategies adopted by organisms in response to the primary determinants of their natural distribution and then the strategies specific to colonization wood and litter.

General concepts: r *and* K*-selection; ruderal, combative and stress-tolerant strategies*

During evolution, different types of selection pressure have resulted in polarization between two sorts of organisms with respect to life span and reproductive commitment. *K*-selected organisms characteristically have a long individual life span and a slow or intermittent commitment to reproduction. The converse applies in *r*-selected organisms (Harper & Ogden, 1970). Analysis of the selection pressures involved reveals that they are of three types: environmental stress (*S*-selection), competitive stress (*C*-selection) and disturbance (*R*-selection). According to one scheme favoured currently by some plant ecologists (Grime, 1979) and fungal ecologists (Pugh, 1980; Cooke & Rayner, 1984) these selection pressures result in stress tolerance strategies (*S*-selected), competitive or combative strategies (*C*-selected; Cooke & Rayner regarded 'combative' as being a more appropriate term for fungi) and ruderal strategies (*R*-selected). *C*-

and *S*-selection can be considered as forms of *K*-selection, whilst *R*-selection is equivalent to *r*-selection. It is important to note that as the primary strategies are part of a spectrum of *behaviour*, they should *not* be used to classify individual *organisms* which may exhibit combinations of the different strategies either at one and the same time or at different times during their life cycle. This proviso aside, behavioural attributes of decomposer basidiomycetes which can be understood within the general context of the three primary strategies will now briefly be addressed, and their relevance to community development pathways outlined.

Ruderal strategies

Ruderal strategies are promoted by disturbance, which can be defined as any sudden environmental event which, either by *destruction* of resident biomass or *enrichment* of the habitat, provides a virgin resource for colonization. These strategies are based on rapid arrival, capture and conversion to fungal biomass of easily assimilable growth substrates, and rapid commitment to reproduction before competitors become established.

Combative strategies

Combative strategies are promoted in undisturbed habitats, relatively free from stress, and in which there is consequently a high potential incidence of competitors. Success therefore depends on the ability to defend domain which has been occupied using the process of 'primary resource capture' or to sequester domain from previous residents via 'secondary resource capture' (Cooke & Rayner, 1984; Rayner & Webber, 1984).

Stress-tolerant strategies

These strategies depend on tolerance of an environmental stress, defined as any more or less continuously imposed feature other than competition, which limits the production of biomass by the majority of organisms under consideration, e.g. extremes of temperature, humidity, aeration and grazing pressure. A special form of stress tolerance, latent invasion, allows sparse development in a habitat where stress prevents colonization by potential competitors, followed by rapid capitalization, i.e. luxuriant mycelial outgrowth from

the previously established inoculum, after a lessening of the stressful conditions. This gives a decisive advantage to the latent invader in primary resource capture.

Strategies and community development pathways

Understanding of successional changes in fungal communities has been obscured by overemphasis of floristic changes and lack of appreciation of the wide variety of factors which can result in replacement of one individual, population or community by another (Rayner & Todd, 1979; Cooke & Rayner, 1984; Rayner & Webber, 1984). One approach to rationalizing the complex sequences of events is to consider the available pathways along which community development may be channelled by four major determinants of community change: stress-aggravation, stress-alleviation, intensification of combat, and disturbance. A simplified scheme based on the behavioural strategies which will predominate in communities developing under a range of circumstances is presented in Fig. 2.

Colonization strategies of wood decay basidiomycetes in standing trees

To the wood-decaying basidiomycete, the standing tree represents an enormous reserve of food. However, access to this reserve is impeded by all those physical and chemical factors which ensure proper functioning of the tree. Five distinctive colonization strategies can be identified whereby these factors are overcome, circumvented or tolerated. These are: unspecialized opportunism, active pathogenesis, specialized opportunism, heartrot, and desiccation tolerance. All are based on the unsuitability of functionally intact sapwood as a habitat for mycelial growth (Rayner, 1986*b*; Rayner & Boddy, in press). Before outlining the essential features of these colonization strategies, the same proviso made with respect to general strategies must be made, to the effect that they represent identifiable nodal points in a continuum of behaviour.

Unspecialized opportunism

This is exhibited when normally inaccessible sapwood is made suddenly available for colonization by injury or rapid death of the bark. Damage represents both a disturbance and an elimination of a major

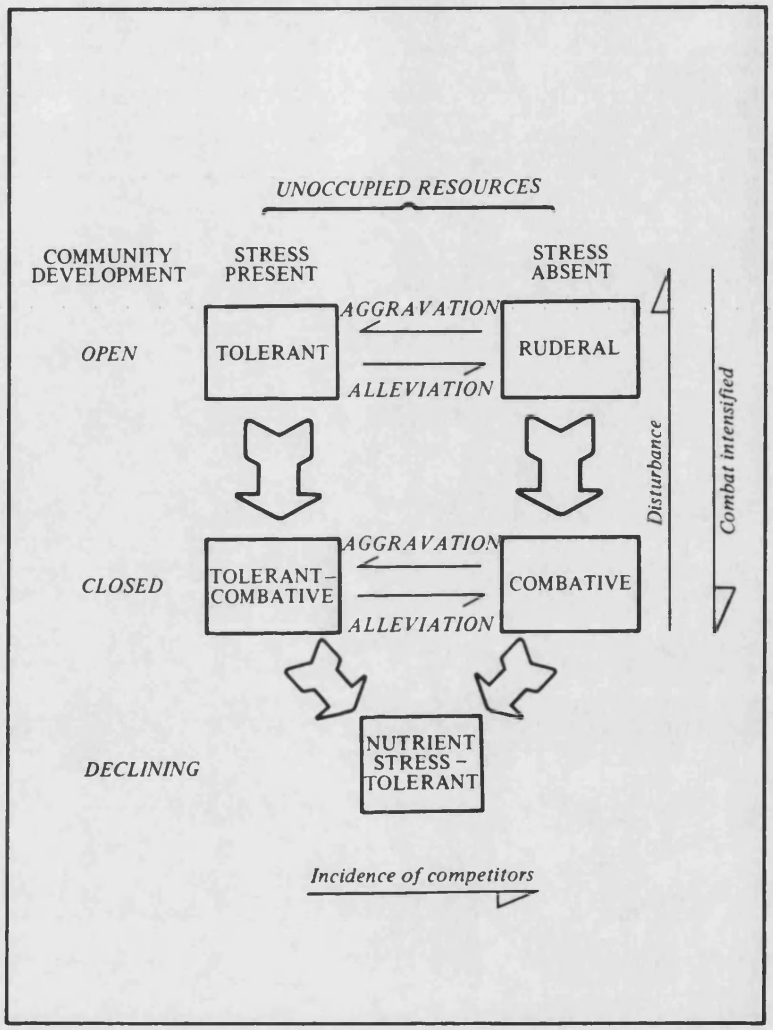


Fig. 2. Diagram of possible community development pathways from colonization of a totally unoccupied resource, through an open community stage with still unoccupied resources available for primary capture, to a closed community with all primary capture of domain completed. The pathways culminate in a declining stage characterized by severe nutrient stress. In the absence of competitors, developing tolerant communities may progress directly to declining tolerant communities without an intermediate combative stage. (From Rayner & Webber, 1984.)

stress barrier. Hence a pattern of community development can be expected in which there is initial selection of organisms with ruderal attributes followed by establishment of combative communities. This is consistent with numerous observations of the colonization of

wounded angiospermous trees by pioneer communities dominated by non-basidiomycetous fungi and bacteria causing discoloration but not decay, or by basidiomycetes such as *Chondrostereum purpureum* which cause little decay, reproduce rapidly and are readily replaced (Mercer, 1982). True decay fungi, such as species of *Coriolus, Bjerkandera* and *Stereum*, which do not exhibit strong selectivity for particular types of tree, attain dominance at a later stage. However, whilst such colonization sequences resemble those depicted in Fig. 2, they have also been interpreted in terms of progressive breakdown of the defences of living tree tissues (Shigo, 1979).

Limitation of colonization following wounding has been ascribed to active host defence (Shigo, 1979, 1984). However, the view favoured here is that repair mechanisms which seal off damaged from functionally intact tissue are really responsible (Boddy & Rayner, 1983*a*; Rayner, 1986*b*). The immediate effect of injury or bark death is exposure of sapwood tissues to air and consequent drying due to cavitation and evaporation. The penetration of air depends on the location, timing and severity of damage, and defines exactly those tissues which become available for colonization by opportunists. Penetration by air is, in turn, dependent on the distribution of void space resulting from wood anatomy, on whether water columns are under tension or pressure, and on the rate of sealing damage by production of gums, resins, tyloses etc. For example, deep wounds of the trunk commonly give rise to colonization zones in the form of two inverted wedge-shaped cones which extend mostly upwards rather than downwards, in autumn rather than in spring, and in older rather than in recent sapwood (Coutts, 1976; Leben, 1985; Rayner, 1986*b*). The sealant zones delimiting the colonized regions are of two types: 'barrier' zones between wood extant at the time of damage and wood formed subsequently, and 'reaction' zones within tissues extant at the time of damage (Shain, 1979).

Active pathogenesis

The distinction between active pathogens and opportunists is that the latter rely on other agents to alleviate unfavourable conditions in functionally intact sapwood while the former do so themselves. They achieve this by killing living tissues, notably in the cambium and medullary rays, and by destroying pit membranes, both of which help to generate and extend regions affected by cavitation. Unlike parasites of non-woody plants or plant parts, the pathogenic activities of these fungi can be seen as a means of preventing maintenance

of the hostile microenvironmental conditions which bar their way to already dead xylem, rather than as a mode of nutrition in itself.

Critical to the success of active pathogens is the establishment of a sufficient inoculum base from which an attack can be made on the host tissues before the defence and/or repair mechanisms of the latter deny access to the fungus. Establishment of this base may be achieved by a variety of means, including initial exploitation of other colonization strategies, notably heartrot, wound colonization and specialized opportunism (see below). However, the primary involvement of an active pathogenic mechanism is at least certain in one major case, the ectotrophic infection of living roots. As its name implies, this habit involves the superficial spread of a mycelial front over or within the bark, in advance, sometimes by 1 m or more, of occupation of the wood cylinder itself. It is the hallmark of several economically important tree pathogens including *Armillaria* spp., *Heterobasidion annosum*, *Phellinus noxius*, *P. weirii*, *Rigidoporus lignosus*, *Inonotus tomentosus* and several *Ganoderma* spp. Whilst Garrett (1970) regarded the ectrotrophic habit as a mechanism for 'diluting out' host resistance, it is better seen as a way of establishing an effective, extensible inoculum from which outer host cells, including cambium, are killed, hence opening the way to the wood cylinder. It is also clear that this mechanism can only operate in environments such as soil or litter where the mycelial front can be supplied by connections with already colonized material (see below).

Specialized opportunism

The basis for this strategy is that particular version of stress tolerance referred to earlier as latent invasion. Accordingly, fungi using this strategy capitalize on alleviation of microenvironmental stress brought about by factors other than their own activities, in which sense they are opportunistic, whilst being in a position so to capitalize by first becoming established under stressful conditions, hence being specialized. The involvement of this strategy in establishment of decay in trees has not yet been demonstrated unequivocally, but there is strong circumstantial evidence for it, especially in relation to the occupation of domain by individual genotypes (see below). Basidiomycetes believed to possess this strategy include a wide range of species whose pathogenicity has never been established directly, but which often exhibit considerable preference (selectivity) for trees of a particular type. Examples include *Piptoporus betulinus* on *Betula*, *Oudemansiella mucida* on *Fagus*, *Peniophora limitata* on

Fraxinus, and *Peniophora quercina* and *Stereum gauspatum* on *Fagus*. Host-selective xylariaceous ascomycetes in *Hypoxylon* and related genera exhibit similar behaviour (Rayner & Boddy, 1986 and in press).

A general mechanism underlying specialized opportunism may be limitation of water supply to functionally intact sapwood arising from internally or externally imposed stresses: that is, stress to the tree results in stress-alleviation for fungi and predisposes the tree to 'infection' by specialized opportunists. Drought stress is probably a particularly frequent factor allowing establishment of specialized opportunists, which often occur in trunks or branches lacking obvious major wounds which serve as colonization foci for air-borne fungi with other strategies.

Heartrots

Once erroneously regarded as the primary cause of decay in standing trees, the heartrot fungi circumvent the problems of colonizing sapwood by growing in the heartwood, where living cells are absent or rare, and in which there is often a relatively extensive gaseous phase. Nonetheless, conditions in the heartwood are commonly highly stressful, gaseous conditions being far from atmospheric and the tissues commonly being suffused with inhibitory chemicals (extractives), generally phenolics. Accordingly, heartrot fungi frequently exhibit a high degree of host selectivity, grow slowly, persist for many years and lack combative ability against less specialized decay fungi. They are amongst the best examples of pure stress-tolerant strategies in the fungal world.

Desiccation tolerance

When bark or sapwood function is entirely lost from standing trunks or attached branches, the underlying wood experiences fluctuations in moisture content and may dry out. Probably quite a large number of stress-tolerant decay fungi can exploit this situation but at present very little detailed ecological information is available about them. However, their principal adaptations may be expected to include production of mycelia and reproductive bodies capable, for example, by production of chlamydospores or mucilage, of dormant survival in the dry state and rapid resumption of function when wetted. Amongst homobasidiomycetes, *Rhodotus palmatus*, *Peniophora cinerea*, *P. lycii* and *Schizopora paradoxa* are good examples, whereas amongst heterobasidiomycetes, jelly fungi such as *Exidia*, *Tremella*,

Auricularia and *Dacrymyces* are probably able to tolerate fluctuations in moisture availability. Xylariaceous ascomycetes and gelatinous discomycetes, e.g. species of *Bulgaria*, *Neobulgaria* and *Ascocoryne*, probably possess at least partial desiccation tolerance.

Integration of colonization strategies during decay community development in a standing tree

From the foregoing account, a complex picture emerges of the processes by which communities of fungi, including basidiomycetes, combine to bring about the eventual demise and decomposition of the standing tree. At first the emphasis will be primarily on the direct interaction between the fungi and tree, and the variety of ways in which the selectively hostile stress conditions imposed actively or passively by the tree can be countered, alleviated, overruled or by-passed. Invading fungi will hence largely be separated from one another by their differing colonization strategies, so that interfungal interactions will largely be confined to those within or between populations with shared or overlapping strategies.

As time proceeds, and the tree begins to decline, so the emphasis will change. Pathogens in the very act of directly alleviating conditions for themselves will predispose sapwood to colonization by specialized and unspecialized opportunists. Unspecialized opportunists previously confined to regions within the vicinity of damage will begin to extend their domain into previously uncolonized territory. Death and colonization will spread to branches of progressively higher order. Heartrot fungi, previously confined within the central wood cylinder, may begin to encroach outwards, only to be met by newly established residents in sapwood. Dead, exposed limbs may lose so much water that fungal activity within them can only be sustained by species with desiccation-tolerant strategies.

In many parts of the tree the stress conditions which first dictated colonization are progressively alleviated and intensification of combative conditions occurs. The ensuing battles will first involve the resident community of pioneers, with the opportunists perhaps having the edge over the pathogens and heartrot fungi. Later, truly combative fungi may establish themselves and begin to replace the pioneers. From the air these may include, in angiosperms, such species as *Phlebia radiata* and *Coriolus versicolor*. From the soil, combative cord-formers such as *Hypholoma fasciculare* and *Phanerochaete velutina* will invade (see below). The intense decay caused by some of these fungi will, in addition to that caused by active pathogens,

hasten the fall of limbs and the windthrow of trunks. The stage is then set for new cycles of invasion and interactions on the woodland floor.

Strategies in felled or fallen wood and litter

To reiterate, apart from roots, neither wood nor litter will normally arrive at the woodland floor in an uncolonized state, although the nature of the resident microbial communities in each may be very different. Consequently, basidiomycetes which actively decompose wood and litter in this location exhibit the capacity either to defend domain which they have captured before or soon after fall, or to gain access to domain by secondary resource capture. The difference between defensive and attacking strategies is often reflected in a further major distinction in behaviour between resource-unit restriction and non-restriction (Cooke & Rayner, 1984; Rayner, Watling & Frankland, 1985). Mycelia of unit-restricted fungi are confined to individual resource-units, be these petioles, fruits, twigs, or branches. Mycelia of non-unit-restricted fungi are not confined to individual units of wood or components of litter, but can migrate between these units.

Defensive strategies and unit restriction

With respect to wood, it is clear that some decay fungi can persist for many years, having first colonized the tree while it was still standing, and without growing out to colonize new domain. This applies best to certain xylariaceous ascomycetes, such as *Daldinia concentrica* in *Fraxinus*, so that whilst some species of *Pleurotus, Ganoderma, Piptoporus* and *Phellinus* can persist, many of the basidiomycetes colonizing before fall appear to be rather readily replaced thereafter. Certain species of *Coriolus* and *Stereum*, e.g. *C. versicolor* and *S. hirsutum*, which colonize cut or broken wood primarily by air-borne spores, although capable of replacing pioneers appear to be primarily defensive thereafter, and may persist for several years.

With regard to litter, good examples of unit-restricted basidiomycetes are provided by the genera *Marasmius* and *Mycena sensu lato.* Many exhibit a high degree of selectivity, e.g. *Marasmius buxi* on *Buxus* leaves, *Marasmius hudsonii* on *Ilex* leaves and *Mycena strobilina* on *Pinus* cones (Rayner, Watling & Frankland, 1985). How they become established is unclear, but it may be that some sort

of latent invasion mechanism, allowing establishment before fall, is involved: otherwise they must arrive, presumably as spores, and be able to establish by replacing the previously resident microflora. The remarkable tropical agaric *Crinipellis perniciosa* serves as a useful illustration of principles: basidiospores infect developing cocoa (*Theobroma cacao*) tissues, eliciting formation of abnormal growths (brooms) which become ramified by a non-culturable mycelium; following death of the broom, a culturable mycelium develops which produces crops of fruit bodies for up to several years (Hedger, 1985; Wheeler, 1985).

Attacking strategies and non-unit restriction

The life of unit-restricted fungi on the woodland floor, both in wood and litter components, is ultimately often limited by the presence there of highly combative non-unit-restricted fungi. The role of the latter is to scavenge the woodland floor, consuming all appropriate resource units that fall within their path, then to move on inexorably until they meet another equally or more combative non-unit-restricted individual of the same or a different species. However, relating to the distinction between wood and litter described earlier, the form of the mobile mycelial units differs markedly according to habitat type. Wood inhabitants ramify the woodland floor in the form of linear mycelial aggregates, mycelial cords and rhizomorphs, which interconnect between spatially discontinuous woody resource units. Litter inhabitants produce diffuse mycelia which ramify the litter layer as a whole: these are the fairy-ring formers, notably species of *Clitocybe*, *Collybia*, *Marasmius* and *Mycena* (Cooke & Rayner, 1984; Thompson, 1984). Intermediate between the wood and litter non-restricted fungi just described are various species of *Marasmius, Marasmiellus* and *Crinipellis* which migrate between litter components by rhizomorphic growth, and in humid environments such as tropical rain forest even become epiphytic in intact tree leaves and shoots whilst still attached (Hedger, 1985).

Ruderal strategies

Ruderal strategies on the woodland floor can only be pursued if some form of disturbance occurs. In the case of wood, this is very common, especially in managed woodland or forest, since cutting or felling of trees is an effective means of disturbance. Sequences of colonization along the lines depicted in Fig. 2 are thus promoted,

beginning with predominance of ruderal communities relatively inactive in decomposition, encompassing such basidiomycetes as *Schizophyllum commune*, *Chondrostereum purpureum*, *Corticium evolvens*, *Stereum sanguinolentum* and *Flammulina velutipes*. Combative, active decay species then become dominant including primarily defensive species of *Stereum* and *Coriolus* and non-restricted cord-forming species such as *Hypholoma fasciculare*, *Phallus impudicus*, *Tricholomopsis platyphylla* and *Phanerochaete velutina* (Coates & Rayner, 1985*a*, *b*, *c*).

Animal activity and fire represent other causes of disturbance. *Coprinus* spp. probably include some of the best examples of ruderal litter-inhabiting basidiomycetes.

Stress-tolerant strategies

Replacement of previous mycofloras, or components thereof, need not always imply superior combative ability of succeeding individuals. As indicated in Fig. 2, stress aggravation, e.g. resulting from nutrient depletion or desiccation, can also be important. However, unit and non-unit restriction have been emphasized in this section because of their implications for the relation between developmental regulation and colonization strategies.

DEVELOPMENTAL REGULATION AND COLONIZATION STRATEGIES

It should be clear from the foregoing that a thallus form suitable for one colonization strategy may be quite unsuitable for another. Moreover, the whole process of establishment of domain may involve sequential stages such as arrival, establishment of an inoculum base, primary resource capture, resource exploitation, defence, and secondary resource capture, all in an environmental setting which may be constantly changing via stress alleviation, stress aggravation, disturbance and intensification of combat. It would indeed be a challenge to account for all this on the basis of a view of the basidiomycete mycelium as little more than an assemblage of protoplasm-filled duplicating units (hyphal tips) between which there is little or no intercommunication or functional differentiation. On the other hand, the variability of behaviour ('mutability') of mycelia has always been recognized by mycologists, but regarded as mysterious, if not treacherous and frustrating, by experimentalists.

When set within its true, ecological context, the intrinsic variability of mycelial growth becomes not frustrating but the explanation for the versatility in development which is required for establishment in spatiotemporally heterogeneous environments. A new picture emerges of the vegetative thalli of basidiomycetes, and indeed other fungi, as entities which can adopt a variety of alternative forms ('modes' as Gregory (1984) called them) conferring different functional properties. Hence individuals with different colonization strategies can adopt suitably distinctive developmental patterns, whilst the same individual may be able to switch from one pattern to the other as circumstances dictate. Moreover, fungi with narrow ecological niches may become fixed into particular developmental patterns, whilst greater plasticity will be retained by those with broader niches.

In trying to bring some order to the chaos of observed variability in development of fungal thalli, Rayner & Coates (in press) postulated the existence of at least five distinctive sets of alternative modes of morphogenesis. They suggested further that these alternatives are modulated genetically by a series of superimposable switch mechanisms which are *cued* by a wide variety of endogenous and exogenous stimuli. The nature of the exogenous stimuli is presumably dependent on the environmental signals which would normally be encountered by the fungi concerned. Rayner and Coates detailed possible criteria aiding distinction of simple mutations and direct environmental effects on metabolic functioning from the proposed switch mechanisms. Therefore, rather than elaborating on these mechanisms, our approach here will be to focus on the ecological implications of the alternative states which Rayner & Coates proposed.

Determinate/cellular – interdeterminate/filamentous transitions

Conversion from determinate to indeterminate morphogenesis occurs at spore germination and in reverse at sporogenesis. A growing number of fungi, including many basidiomycetes, are also known to be able to switch between unicellular (yeast-like) and mycelial forms. This capacity has naturally aroused much interest, but the accompanying neglect of other dimorphisms is epitomized by the frequent use of the term 'dimorphism' to cover only the specific case of mycelial–yeast dimorphism (Stewart & Rogers, 1983).

Discussion of cellular–filamentous transitions in an ecological context can thus apply to two distinct issues: the relative merits of spore

production *versus* mycelial spread in dissemination, and of yeasts *versus* hyphae in primary resource capture. In both cases, the relative merits of the cellular and filamentous modes can be approximated, respectively, to the demands of *r*- and *K*-selection.

The differing characteristics of spores and mycelium as means of arrival at resource surfaces by basidiomycetes has been discussed in some detail by Rayner, Watling & Frankland (1985) who pointed out that the greater the reliance on spores, the greater will be the tendency for resource unit restriction. To summarize, spores can be produced in large numbers but, unless associated with a vector, they lack means of ensuring their arrival at suitable resources other than by random selection. Furthermore, colonization from spores will be effected from localized foci, opportunities for input of water and nutrients will be limited, and buffering against hostile influences at the resource surface will be absent. Hence spores lack inoculum potential (cf. Garrett, 1970).

Arrival by mycelium is a particular feature of those non-unit-restricted basidiomycetes colonizing wood or litter on the ground or in humid aerial environments. Much greater inoculum potential can be brought to bear by the mycelium, enhancing the ability to replace residents or overcome stress barriers. Colonization is not limited to localized foci, nutrients and water may be imported from an already established food base and, by formation of compacted structures such as cords (see also later), buffering against hostile abiotic conditions can be achieved.

Because the hyphae grow from their tips, mycelial growth can be highly polarized – to a degree depending on branch angle and frequency, which are controlled by separate switch mechanisms (see below). As a result, considerable directionality can be achieved during growth between discontinuously distributed nutrient depots, so conserving energy by reducing wastage of biomass due to growth in an unproductive direction. This is illustrated by the behaviour of *Hypholoma fasciculare* during growth between woody resource units in soil (Fig. 3*a–h*). This figure demonstrates powerfully the remarkable ability of the mycelium to behave as a co-ordinated unit (Dowson, Rayner & Boddy, 1986): as an army it sends out scouting parties, establishes lines of communication, brings in reinforcements by redirecting the movements of its troops, conquers domain, establishes bases, and moves on.

The conservation of growth polarity illustrated in Fig. 3 for a mycelial system which is compacted into mycelial cords (see also

below) also has considerable bearing on those diffuse mycelial systems of litter-inhabiting basidiomycetes which form fairy rings. The annular shape of fairy rings has always been difficult to explain, since although build-up of allelopaths and nutrient limitation have been suggested as reasons for the absence of mycelium from the central regions, leaching and enrichment by litter fall should alleviate these stresses. Cooke & Rayner (1984) suggested that the annulus was due to the establishment of a source–sink relation conserving polarity between the trailing edge and mycelial front, and this now seems a satisfactory explanation. In field experiments with *Clitocybe* rings, we excised segments of the mycelium and reorientated them within, outside or behind the annulus. Within the annulus the reoriented mycelial front ceased growth; outside or behind the annulus, growth continued with conserved polarity (Dowson, Rayner & Boddy, unpublished).

Once arrival has been effected, propagation of unicells and mycelial proliferation have complementary advantages and disadvantages. Hence unicells can be well equipped for rapid capture and conversion to biomass of easily assimilable substrates, for dispersion in mobile media, and for tolerance of adverse water potential, aeration and nutrient limitation (see also Nedwell & Gray, this volume). On the other hand mycelia are well equipped for conquest of fixed spatial domain, breakdown of refractory substrates via extracellular enzyme action, functional compartmentalization, and penetration or obviation of physicochemical barriers.

Just as in certain fungal pathogens of insects and vertebrates, the facility to switch between unicellular and mycelial morphogenesis may be expected to be critical to successful establishment of many wood- and litter-inhabiting basidiomycetes. However, the full extent to which this applies is not yet clear. It is established that production of a unicellular stage is important in the transmission of wood-decaying species of *Stereum* and *Amylostereum* by wood wasps, and the chlamydosporic mycelia of *Rhodotus palmatus* and *Schizopora paradoxa* seem likely to be important in their desiccation-tolerant strategies (Rayner & Boddy, 1986). But the greatest opportunities for deployment of a unicellular stage in establishment would seem to be within the sapstream of standing trees; this is after all the mechanism by which wilt fungi such as *Ceratocystis ulmi* and *Verticillium* spp. gain access to the vascular tissues. Such a mechanism would readily provide the basis for a specialized opportunist strategy but unfortunately it has yet to be demonstrated, for certain, in practice.

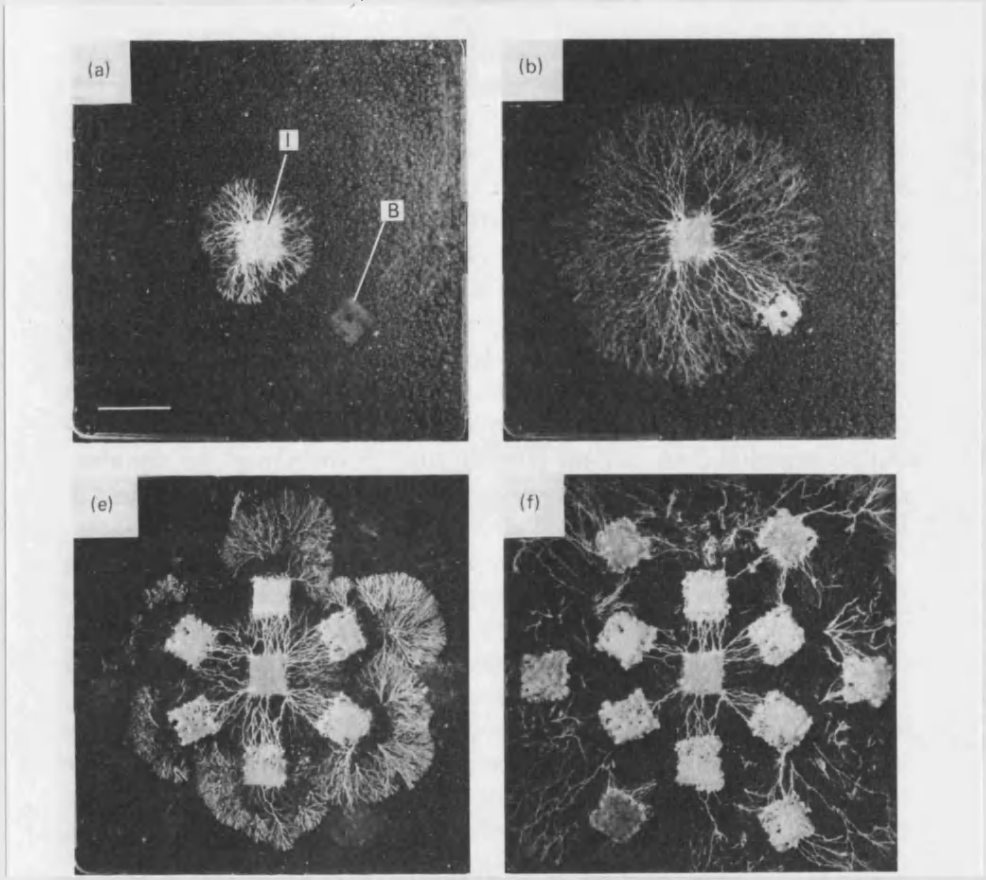
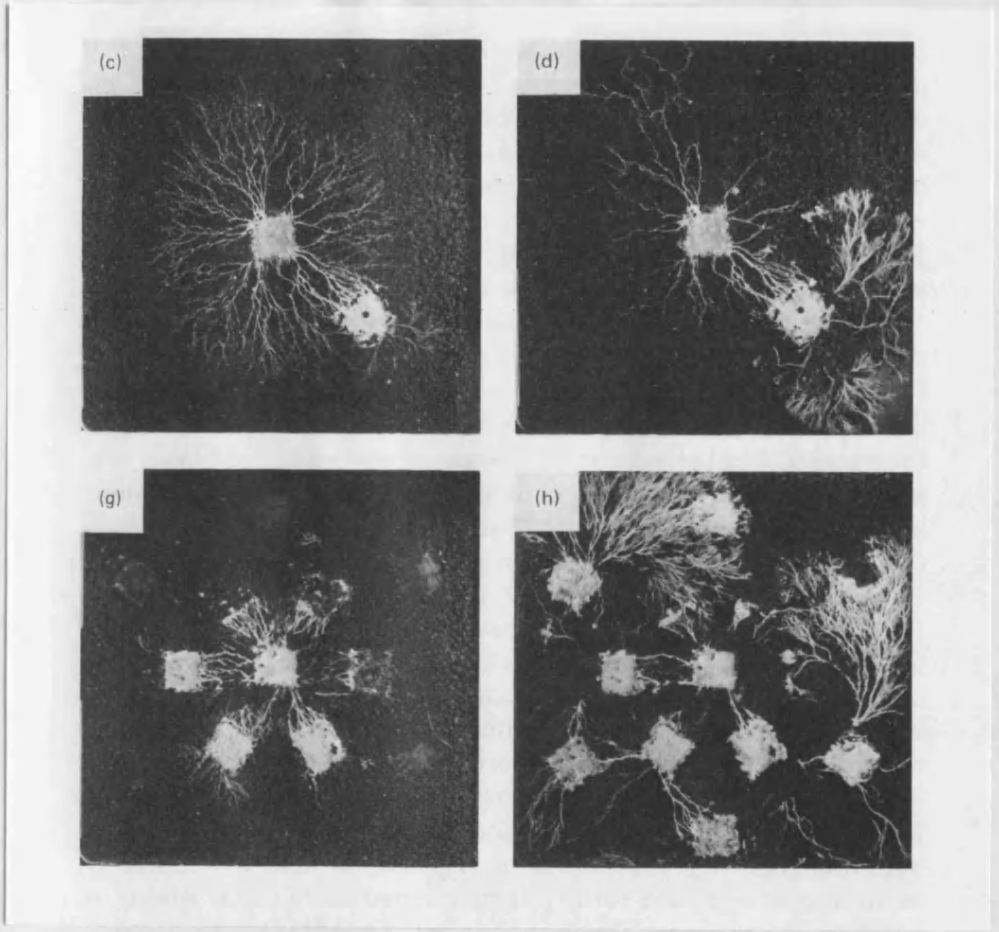


Fig. 3. Growth of non-restricted, cord-forming mycelium of *Hypholoma fasciculare* through non-sterile soil between woody resource units. (*a–d*) Growth from an inoculum wood block (I) towards an uninoculated 'bait' wood block (B). (Scale bar = 4 cm) (*e*, *f*) Growth from a central inoculum to an hexagonal array of baits, showing symmetrical outgrowth patterns. (*g*, *h*) As (*e*, *f*) but with three baits adjacent to the central inoculum removed after initial contact. Note the cessation of growth where baits have been removed, and asymmetric outgrowth from residual baits to colonize the outermost wood blocks. (Photographs from Springham, 1986).

Wood-inhabiting heterobasidiomycetes, particularly in the Tremellales, are well known to produce yeast phases as part of their life cycle (see below), but the colonization strategies of most of these fungi remain obscure, except for some which parasitize homobasidiomycetes. In homobasidiomycetes, by contrast, where latent invasion mechanisms are strongly implicated, production of yeast phases has yet to be confirmed, although production of conidia, especially by haploid homokaryotic mycelia, is undisputed. However, evidence is beginning to accrue that homobasidiomycetes, including wood-



decaying forms possessing putative latent invasion strategies, may after all be able to produce yeast phases (Rayner & Coates, in press and unpublished; Prillinger, 1984, 1986 and in press), and that phenolics produced by the tree in response to wounding or stress could provide cues for reversion to mycelium (Dowson & Rayner, unpublished). If verified, these observations would have far-reaching implications, but for now they must be considered unsubstantiated.

Alterations in internode length and branch-angle: 'gear shifts'

The switch between unicellular and filamentous growth is in fact just the primary example of how growth resources can be reapportioned for greater or lesser polarity by morphogenetic controls in fungi. Subservient to this is a further system analogous to the gearbox

of a forward-driven motor vehicle which enables selection of different degrees of conversion of engine torque to forward motion. Thus mycelia can adjust their degree of polarity in two main ways: by altering internode length (associated with hyphal diameter and branching frequency), and by varying branch-angle to provide different degrees of alignment of marginal hyphae (Rayner *et al.*, 1985; Rayner & Coates, in press). An equivalent system appears to control polarity of yeast pseudomycelia (unpublished observations).

The existence of gear shifts in basidiomycete mycelia is often evident from patterns of mycelial outgrowth from a germinating spore (Fig. 4*a*) and can become 'fixed' in striking 'slow dense/fast effuse' dimorphisms such as that illustrated in Fig. 4*b*. They also encompass the remarkable behaviour on agar media of certain *Phlebia* spp. in which the mycelial margin is composed of rapidly extending, appressed, coenocytic hyphae, and is followed from behind by a consolidatory phase of septate, aerial hyphae (Fig. 4*c*).

The facility for 'changing gear' has immense ecological implications. For example, a high (fast forward) gear will facilitate exploration and coverage of domain, together with rapid extraction of easily assimilable nutrients (cf. *r*-selection), whilst a low gear may aid in initial establishment of an inoculum base, exploitation of refractory resources, consolidation of territorial gains and stress tolerance (cf. *K*-selection). These principles are illustrated by the alternations between exploratory and consolidatory growth evident during the migration between food bases of *Hypholoma fasciculare* (Fig. 3) as well as the growth form just mentioned in *Phlebia*, where, in addition, the coenocytic hyphae have been found to lack the recognition responses necessary for effective combat (Boddy & Rayner, 1983*b*; *cf.* below).

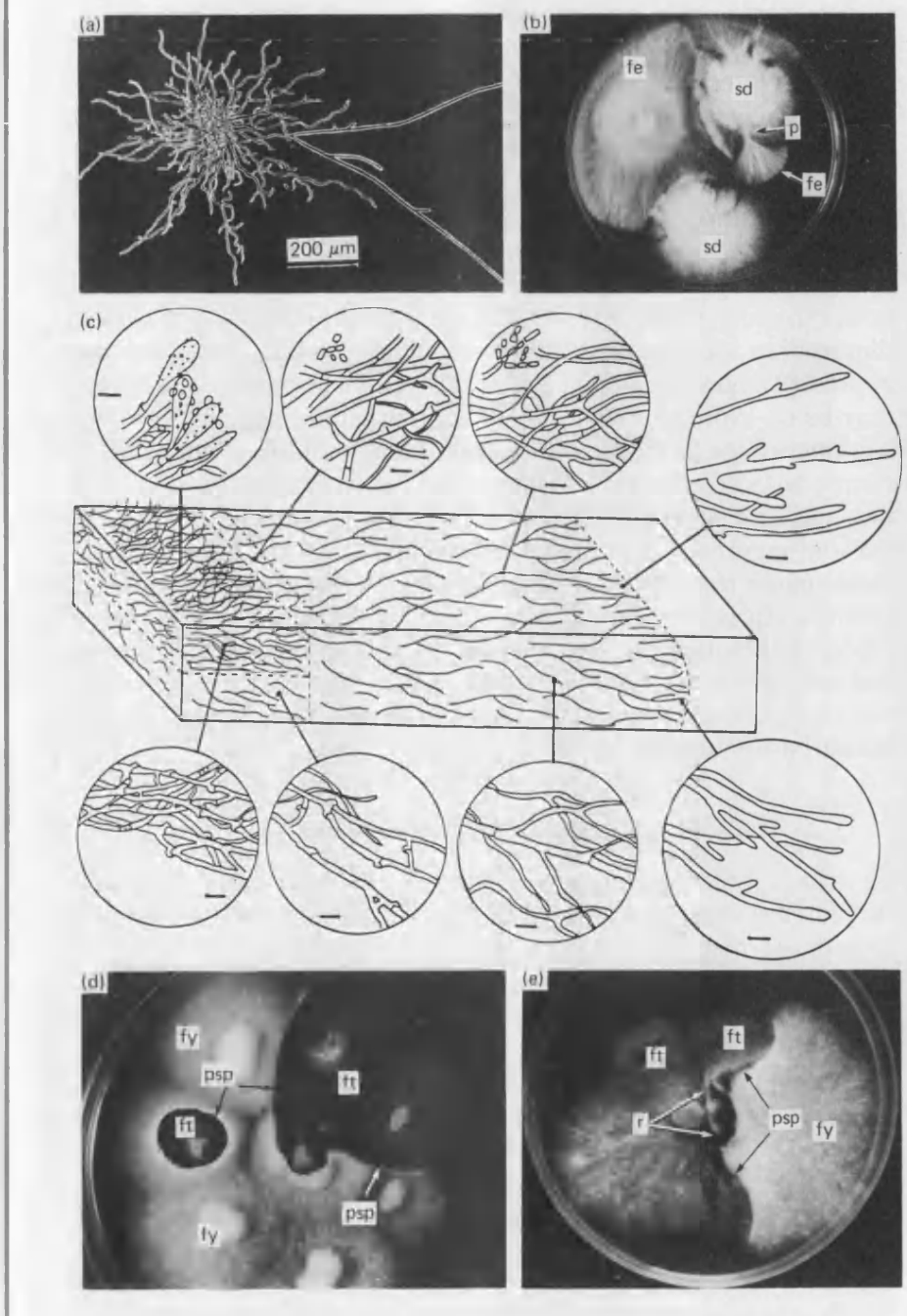
Aerial versus appressed or submerged growth

Production of mycelium which is not in intimate contact with the substratum (referred to here as 'aerial mycelium') acts both as a drain on resources from trophic mycelium, and as a means of freeing growing hyphae from physicochemical constraints within the substratum. Aerial and non-aerial growth may, in different basidiomycetes, be closely coupled, partially uncoupled (resulting in endogenously or exogenously regulated rhythmicity), or largely uncoupled, resulting in distinctive growth phases, dimorphisms or polymorphisms.

Amongst Basidiomycotina, striking aerial–non-aerial dimorphisms occur in the wood decaying members of the Hymenochaetaceae. In *Hymenochaete corrugata*, the two colony forms have similar extension rates, but the appressed form is yellow-brown whilst the aerial form is white (Fig. 4*d*, *e*). Both forms develop on the natural substratum, the appressed type being associated with more-decayed wood (Sharland, Burton & Rayner, 1986), a feature which is of particular interest because only this type possesses the tyrosinase and laccase activities which are associated with ligninolytic activity and secondary metabolism (P. R. Sharland, personal communication; *cf.* Ander & Eriksson, 1978; Kirk & Fenn, 1982). A very similar dimorphism occurs in *Phellinus tremulae* except that here the appressed pigmented form has a slower extension rate (i.e. there may be co-expression of the slow dense switch) and can grow over a greater range of temperatures than the aerial form (Hiorth, 1965; Niemelä, 1977). Similar behaviour also occurs in *Rigidoporus lignosus*, the root pathogen of rubber, with the interesting implication that the superficial ectotrophic mycelium may be in a different functional mode from the laccase-producing mycelium within the wood cylinder (Boisson, 1968; Geiger, Nandris & Goujon, 1976). This places the ectotrophic infection habit in an entirely new perspective, and indeed may have general implications regarding the switch from mycelial arrival and establishment to exploitative growth in lignocellulosic basidiomycetes.

Compacted versus diffuse morphogenesis

At critical times during mycelial development in basidiomycetes, the initial divergent growth of hyphae is superseded by convergent growth, perhaps mediated by positive autotropisms (Ainsworth & Rayner, 1986) resulting in hyphal fusion (*cf.* Fig. 4*c*) and aggregation. By such means are generated the compacted, tissue-like structures referred to generally as plectenchymatous, e.g. fruit bodies, sclerotia, stromata, pseudosclerotia, rhizomorphs and mycelial cords. The exact form of these plectenchymatous structures is probably dictated principally by how localized or generalized is the compaction process, and by the mycelial form on which it operates (Rayner & Coates, in press). Hence, generalized expression of this phenomenon within a plane of diffuse mycelium probably accounts, together with proliferation of branching, for the formation of the crust-like plates which, when formed within substrata, delimit what have been termed


(a)
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pseudosclerotia (Campbell, 1933). Interestingly, juxtaposition of the aerial and non-aerial forms in *Hymenochaete corrugata* results in formation of such a pseudosclerotial plate (Fig. 4*d*, *e*), whose primary ecological function seems to be defensive against potential combatants and protective against adverse abiotic environmental factors, especially desiccation.

Compaction of outwardly extending, collaterally aligned hyphal systems results, by contrast, in formation of linear organs such as mycelial cords and rhizomorphs. Depending on the degree of polarity of these structures, a spectrum of types can be formed, from the apically dominant true rhizomorphs such as those of *Armillaria* spp., to apically diffuse forms (Fig. 5). The extreme polarity of *Armillaria* rhizomorphs is implicit in the fact that they exhibit an extension rate an order of magnitude greater than that of diffusely growing hyphae (Rishbeth, 1968). The primary function of these linear organs is as connectives, allowing supply from a food base to either a sporophore or an actively growing mycelial front (*cf.* Fig. 3), whence they commonly contain an internal system of wide 'vessel' hyphae (see Rayner *et al.*, 1985). Although often produced in culture as a result of combative interactions, they are probably not normally directly involved in combat in nature, since systems of different individuals can often interdigitate significantly in soil. However, once at a food base the increased inoculum potential they confer may improve combative ability significantly. Where systems of different individuals or species come into contact, reactions equivalent to somatic incompatibility and hyphal interference often occur.

In *Steccherinum fimbriatum*, cord systems have been found which exhibit a slow dense/fast effuse switch, demonstrating further how mechanisms of recognition and developmental regulation characteristic of diffuse hyphal systems can be recapitulated at the level of hyphal aggregates.

Fig. 4. (*a*) Development of a colony of *Coniophora puteana* from a single basidiospore, showing progressive shifts in extension rate of marginal hyphae. (After Kemper, 1937.) (*b*) Outgrowth of mycelia from fruit body tissue of *Hypholoma fasciculare* onto 2% malt agar, showing slow dense (sd)/fast effuse (fe) dimorphism and origin of fast-effuse sectors by 'point growth' (p). Compare with outgrowth patterns in soil shown in Fig. 3. (*c*) Colony characteristics of a dikaryotic culture of *Phlebia radiata* growing through 2% malt agar, illustrating the marginal zone of coenocytic, non-anastomosing hyphae which is superseded by septate, anastomosed hyphal growth with clamp-connections. Scale bars = 10 μm. (After Boddy & Rayner, 1983*b*.) (*d*) Subcultures from a single colony of *Hymenochaete corrugata* which have grown out in 'flat' (ft) and 'fluffy' (fy) non-aerial and aerial forms, the two colony types interacting to produce a pseudosclerotial plate (psp). (From Sharland, Burton & Rayner, 1986.) (*e*) Rejection reaction (r) between two different heterokaryons of *H. corrugata* and associated changes in morphogenesis (*cf.* Fig. 4*d*). (From Sharland, Burton & Rayner, 1986.)

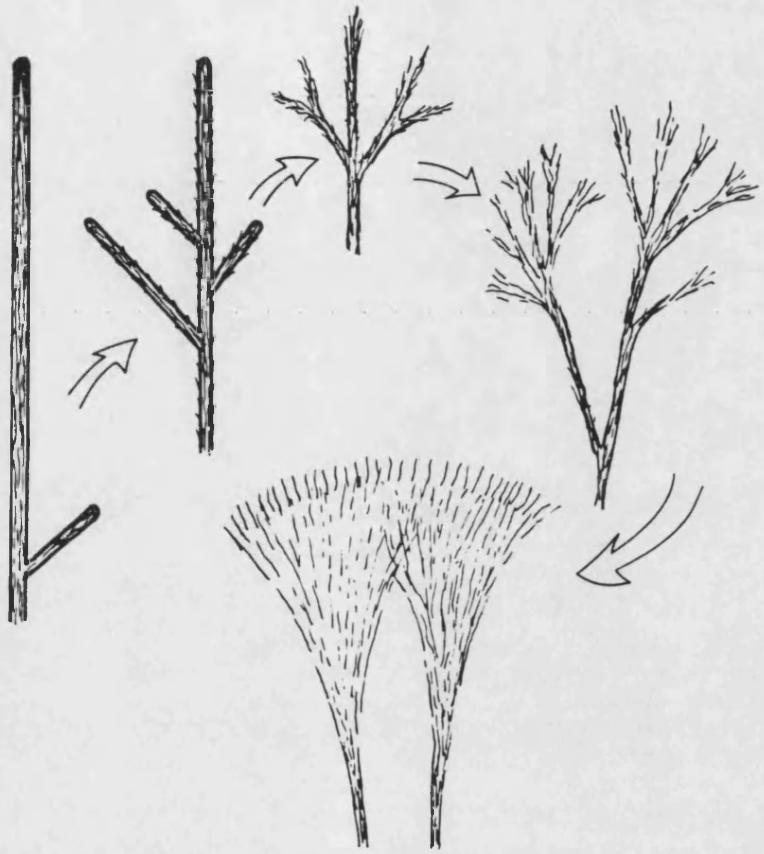

Fig. 5. Diagram illustrating the spectrum of compacted mycelial outgrowth patterns resulting in production of linear organs. Progression from strongly rhizomorphic outgrowth (far left) to diffuse outgrowth followed by consolidation (bottom centre) is associated with loss of apical control over extension of marginal hyphae resulting in increased branching and loss of apical coherence. (From Rayner *et al.*, 1985.)

Juvenility and senescence

In addition to possessing a 'gearbox', basidiomycete mycelia also appear to possess a braking system, enabling their potentially infinite capacity for growth to be brought to a halt. Evidence for this system can be seen in Fig. 3 where contact with a 'bait' results in the entire margin of initial exploratory growth being brought to a halt. Activation of the braking system may generally be a prequisite for redirection of growth resources prior to adoption of a new morphogenetic mode. Indeed it has been suggested that the periodic fruiting of fairy-ring fungi may be conditioned by an endogenous switch of this

sort: since these non-unit-restricted fungi do not encounter a boundary to the resources they occupy, some such mechanism is required to cue the transition from vegetative to reproductive growth (Lysek, 1984).

So what is the basis for this braking system? There is increasing evidence that this may involve elicitation of programmed 'senescence' pathways associated with activation of phenoloxidase systems and melanization; in certain ascomycetes there is further evidence for involvement of cytoplasmic determinants under the control of nuclear genes (Daboussi-Bareyre, 1980; Esser *et al.*, 1984).

The association with phenoloxidase activity and melanization recalls the behaviour of certain aerial–non-aerial dimorphic forms discussed earlier, notably *Hymenochaete corrugata*, where the formation of a melanized pseudosclerotial plate (Fig. 4*d*, *e*) now assumes a special significance. A common mechanism relating damage, phenoloxidase systems, senescence, melanization, induction of plectenchyma formation, sporogenesis and rejection responses (see below) now seems to be emerging (Rayner & Coates, in press; see also Ross, 1985). This would revolutionize our understanding of the behaviour of basidiomycete thalli in nature.

RELATION OF RECOGNITION SYSTEMS TO COLONIZATION STRATEGIES AND MYCELIAL DOMAINS

As indicated earlier, powerful recognition and response systems occur in basidiomycetes which condition acquiescence to, or rejection or acceptance of, self- and non-self within species. The existence of these mechanisms may further relate to the outcome and mechanisms of combative interspecific interactions (Rayner, 1986*a*). The rejection mechanisms are now much more widely known than a decade ago, and are commonly referred to as somatic or vegetative incompatibility. They are proving a valuable tool in analysing population structure (Brasier, 1984; Rayner & Boddy, 1986) because of the often easily observed demarcation zones between different genotypes both in culture (see Fig. 4*e*) and in nature (Fig. 6*a*). Evidence is also accumulating that both rejection and acceptance responses involve modulation of the developmental switches described in the previous section, the rejection responses for example involving activation of the senescence pathways (Rayner & Coates, in press; see

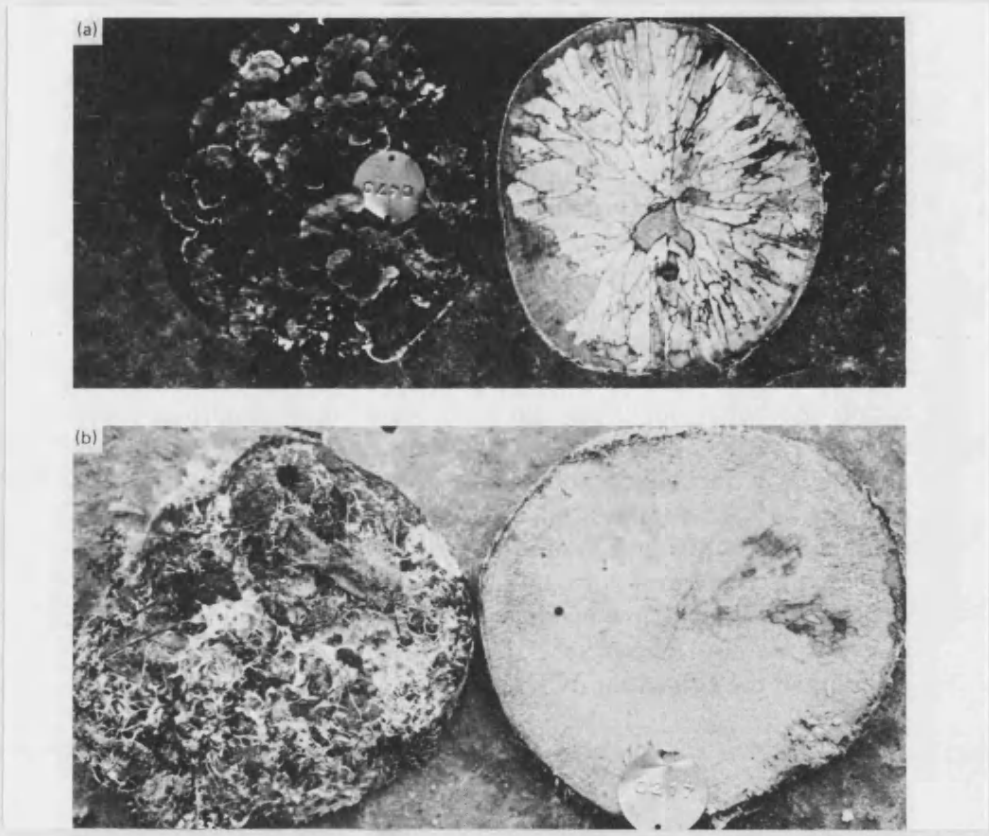

Fig. 6. Typical colonization patterns of upper (*a*) and lower (*b*) cut surfaces of beech (*Fagus sylvatica*) logs placed upright with their bases buried in the litter of a deciduous woodland. Numerous genets of *Coriolus versicolor*, delimited by narrow dark interaction zone lines in the decayed wood, are present near the upper cut surface, whilst a single genet of the cord-forming *Tricholomopsis platyphylla* has virtually sole occupancy of the wood adjacent to the lower surface. (From Coates & Rayner, 1985c.)

Fig. 4*e*). However, here only the ecological significance of recognition responses in relation to colonization strategies will be considered in any depth.

Outcrossing versus non-outcrossing strategies

Outcrossing, that is sexual conjugation followed by diploidization and meiosis between genetically different haploid homokaryotic lines, results in the production of variable basidiospore progeny from a single basidiocarp. Hence the degree of genetic variability within decomposer basidiomycete populations will be determined by the degree to which outcrossing occurs. This in turn can be regulated

in two ways: (1) by the extent to which sexual or asexual mechanisms are primarily responsible for propagation of the population; and (2) by interconversion between essentially apomictic and heteromictic, i.e. non-outcrossing and outcrossing, life cycles.

Sexual versus asexual propagation

Where a mating (= homogenic incompatibility = outcrossing = heterothallic = heteromictic) system is functional within a population, then the balance between basidiosporogenesis and other (asexual) modes of propagation will be decisive in ultimately determining the dynamics and structure of that population. Hence, asexual propagation will result in proliferation of particular discrete genotypes as either spatially discontinuous clones or spatially contiguous individual mycelia (henceforth the term 'genet' will be used to describe such population sub-units, following the recommendation of Brasier & Rayner, in press). Conversely, predominance of sexual reproduction will result in a spatiotemporally heterogeneous population, comprising many different genets.

Asexual propagation can involve either mitotic production of discrete propagules – spores, microsclerotia, sclerotia, etc. – or mycelial spread. Relatively little is known about the role of asexual propagules in the population dynamics of decomposer basidiomycetes. Although many species do produce conidia, these are by no means as prolific as in many ascomycetes, and they are commonly haploid – consequently any homokaryotic mycelia established from them are likely to become converted to heterokaryons by mating (see below). Chlamydospores and sclerotial bodies, by contrast, may more commonly be heterokaryotic but they would principally help to ensure survival of a genet in a particular location, rather than facilitating its spread to others. However, the distribution of individual genets of *Athelia rolfsii* on Californian golf greens is indicative of dissemination of sclerotia during cultivation (Punja & Grogan, 1983).

By contrast with asexual popagules, there is little doubt that mycelial spread is, and has been, fundamental in the proliferation of individual genets of non-unit-restricted wood and litter-decomposing basidiomycetes over considerable areas of ground. Hence genets occupying more than a hectare of ground have been detected in rhizomorphic or cord-forming wood decomposers, including species of *Armillaria* (Korhonen, 1978; Anderson *et al.*, 1979; Thompson & Boddy, 1983), *Tricholomopsis platyphylla* (Thompson & Rayner,

1982), and *Phanerochaete velutina* (Thompson & Boddy, 1983). Somewhat smaller genets have been detected in the root pathogens *Heterobasidion annosum* (Chase & Ullrich, 1983; Stenlid, 1985) and *Phellinus weirii* (Childs, 1963) which depend more on root–root contacts for establishment of their ectotrophic infections than on migratory mycelium. On the basis of current evidence, fairy rings appear generally to represent extensive, stable, individual genets, at least in *Marasmius oreades* (Burnett & Evans, 1966; R. C. Aylmore, personal communication), *Clitocybe nebularis* and *C. flaccida* (Dowson, Rayner & Boddy, unpublished). In *C. nebularis*, isolates of the same somatic compatibility type were obtained from different fruit bodies in the same ring some 50 m apart along the circumference.

With respect to establishment from mycelium or basidiospores in individual woody resource units, Fig. 6(*a*, *b*), showing colonization of cut beech (*Fagus sylvatica*) logs, is instructive: mycelial colonization is associated with formation of a spatially extensive individual genet, whereas basidiospore colonization is associated with numerous discrete genets. Moreover, Coates & Rayner (1985*a*, *b*) have demonstrated that artificially high basidiospore inoculum loads can restrict the size of the domains of individual genets to such a degree as to restrict markedly the size – and even the production – of fruit bodies, as well as decay rates.

These colonization patterns in cut logs, however, illustrate only the sort of population structure consequent upon enrichment disturbance. There is growing evidence that in the aerial portions of standing trees the structure of decay populations, originating presumably following arrival as propagules and establishment by strategies other than unspecialized opportunism, is often very different. This is to the effect that individual genets, which are specific to a particular tree, commonly have very extensive domains – often having virtually sole occupancy of a trunk or branch. Correspondingly, they tend to produce the sometimes colossal fructifications which could only be supported by a mycelium commanding a very considerable resource pool (Rayner *et al.*, 1984; Rayner & Boddy, 1986).

The reasons behind the formation of such extensive genets probably varies with respect to the different colonization strategies, but all may be due in some way to the selectively stressful conditions which condition successful establishment. Thus, in heartrots the establishment of extensive individual domains probably takes place (under stress conditions militating against potential competitors) over many years or decades by a process of slow mycelial spread

from a colonization focus. Selection of positionally or otherwise advantaged genets as colonization proceeds deeper into the wood may further reduce the incidence of intraspecific competition.

In the case of some specialized opportunism strategies, formation of extensive genets (often several metres long) appears, in contrast to heartrots, to occur very rapidly, perhaps within a single growing season. As previously suggested, pre-establishment within the vascular system by means of an easily dispersed inoculum, such as yeast cells, would provide a suitable explanation. An interesting possibility, detected with *Piptoporus betulinus* in *Betula*, is that larger numbers of genets may become established in trees whose root systems are infected by *Armillaria* than in trees predisposed to colonization by other factors (Adams, 1982; Rayner & Boddy, in press). A similar situation has been detected with the ascomycete *Daldinia concentrica* in *Fraxinus*.

'Apomictic' versus 'heteromictic' life cycles

Besides production of asexual propagules such as conidia and vegetative reproduction by means of mycelial spread, another means of achieving dissemination of an individual genet is by eliminating the requirement for conjugation between mating-type-compatible homokaryons from the sexual cycle. This can be effected if field homokaryons acquire the capacity to complete the life cycle, including formation of basidiospores and even meiosis, without themselves becoming heterokaryotized. Such 'non-outcrossing' strategies have been detected in a number of populations of wood-decomposers. Their hallmark is the production of identical homokaryotic basidiospore progeny from naturally collected fruit bodies, which give rise to somatically compatible mycelia. Often non-outcrossing populations become subdivided into several to numerous groups, between which a rejection (somatic incompatibility) response occurs directly, without any intervening heterokaryotic stage. A particular study of non-outcrossing populations has been made in the genus *Stereum* (Ainsworth, in press). In summary, whilst some taxonomic species of *Stereum* have so far been found to be composed of entirely outcrossing populations, e.g. *S. gausapatum* and *S. rugosum*, others contain reproductively isolated outcrossing and non-outcrossing subpopulations, e.g. *S. hirsutum*, *S. sanguinolentum*, and in yet other cases closely related species pairs occur, one outcrossing, the other non-outcrossing, e.g. *S. rameale* and *S. ochraceoflavum*. Preliminary indications, in need of much further substantiation, are that

non-outcrossing is characteristic of populations which are on the edge of their range, (e.g. Scandinavian populations of *S. hirsutum*), or which possess colonization strategies with a strong ruderal element, e.g. *S. sanguinolentum* in Europe.

These indications tie in with the general expectation that *K*-selected populations exposed to heterogeneous and changing biotic or abiotic constraints, e.g. those colonizing genetically variable unwounded standing trees, will tend to preserve variability. By contrast, those colonizing a widely available, effectively homogeneous resource, and which commit rapidly to reproduction, will be based on *r*-selection favouring the proliferation of particular well-fitted genets.

Homokaryon–heterokaryon transitions in outcrossing species

An ecologically neglected facet of the life cycles of outcrossing decomposer basidiomycetes is the occurrence of two distinctive phases, the haploid homokaryotic primary thallus, resulting from germination of basidiospores, and the heterokaryotic (common) or diploid (rare) secondary thallus originating from sexual conjugation between primary thalli. This is obviously vital to considerations of establishment, since where basidiospores represent the principal method of arrival, it will be the primary thalli which may predominantly be responsible for establishment.

This situation assumes even greater significance when it is realized that primary and secondary thalli commonly exhibit fundamentally different patterns of morphogenesis – often corresponding directly to the alternative developmental modes itemized in the previous section.

Hence, homokaryons often develop either as budding, yeast-like forms, which is a characteristic feature of some heterobasidiomycetes, or as 'slow dense' mycelia in which the marginal hyphae have short internodes and wide-angled branching. By contrast, heterokaryons often develop as 'fast effuse' mycelia, with marginal hyphae having long internodes and/or acute-angled branches. Heterokaryons also commonly have an enhanced capacity, compared with homokaryons, to produce compact structures such as mycelial cords, rhizomorphs, pseudosclerotia, pseudorhiza and reproductive fruit bodies (Rayner *et al.*, 1985).

These differences in morphogenetic properties between homokaryons and heterokaryons have sometimes been attributed directly

to the genes controlling mating, i.e. the mating factors. However, exceptions to the general trends just mentioned do occur and, more importantly, these trends also seem very likely to be related to differences in the ecological function of primary and secondary thalli. Thus, depending on the rapidity with which they are likely to be converted by mating into heterokaryons, homokaryons will be responsible to a greater or lesser extent for primary establishment of a colonization base in previously unoccupied habitats. By contrast, heterokaryons will be responsible for secondary extension of the colonization base, combat with other individuals and elaboration of structures by which to exit from resource units. The homokaryon–heterokaryon transition may thus best be regarded as a very important cue, rather than being directly responsible for the morphogenetic changes which accompany it.

In order to establish the ecological importance of homokaryon–heterokaryon transitions, it is important to have available some data concerning the longevity of homokaryotic thalli and the kinetics of their conversion to heterokaryons under natural conditions. Unfortunately few such data exist, but there are some indications that considerable differences may exist between different fungal populations with regard to rates of access and migration through established homokaryotic thalli. In the case of *Coriolus versicolor* and *Flammulina velutipes*, use of homokaryotic thalli as a means of viable trapping of basidiospores from the atmosphere served to demonstrate how readily such thalli would be heterokaryotized following arrival on their surface of spores of complementary mating type (Adams *et al.*, 1984; Williams, Todd & Rayner, 1984). These experiments also served to demonstrate, in a woodland site, how heterogeneous was the basidiospore rain of *Coriolus versicolor*: no mating type repeats were attained during a year's sampling at monthly intervals. In a different experiment, inoculation of homokaryons of *C. versicolor* into logs led to establishment of on average two to three heterokaryons per homokaryon inoculated, it being evident that heterokaryosis was complete within 3 months (Williams, Todd & Rayner, 1981). A similar experiment in which cut beech logs were exposed to a natural air-borne inoculum revealed that whilst homokaryons of *C. versicolor* and *Bjerkandera adusta* could be isolated from surface wood over a full 2 year period, indicating continual recruitment of spores from the atmosphere, at depth homokaryons were never found more than 6 months after initial exposure (Coates & Rayner, 1985*a*).

In a recent series of experiments with the cord-forming species *Hypholoma fasciculare* and *Phanerochaete velutina*, wood blocks permeated with homokaryotic mycelia were placed directly into non-sterile soil, and mycelial cord systems allowed to grow out therefrom. The mycelia were sampled after several months. In *H. fasciculare*, which is readily heterokaryotized, often associated with a marked slow dense/fast effuse switch in laboratory culture, all the mycelia were heterokaryotic and often more than one heterokaryon was formed per inoculum. In *P. velutina*, which is much less readily heterokaryotized in laboratory culture, only around 25% of the mycelia sampled were heterokaryotic, the remainder remaining homokaryotic even though forming quite well-defined cord systems (Dowson, Rayner & Boddy, unpublished).

Clearly, there is much further experimental work needed in this field.

Parasitism as a strategy for establishment and domain capture

As already indicated, colonization of cut or broken wood by basidiospores of such fungi as *Coriolus versicolor* and *Bjerkandera adusta* often result in establishment of populations of genets with individually small domains within the wood (*cf.* Fig. 6). Correspondingly, the fruit bodies of such fungi which habitually occupy small domains tend to be of small size. However, two decomposer basidiomycetes, *Pseudotrametes gibbosa* and *Lenzites betulina*, seem to colonize fallen or cut wood rather than standing trees – yet they possess large domains and correspondingly sizeable fruit bodies.

This at first sight enigmatic situation is resolved by realizing that both *P. gibbosa* and *L. betulina* employ a remarkable establishment strategy not unlike that of strangler figs or temporarily parasitic ants (Rayner, Boddy & Dowson, in press). Thus, associated with lack of recognition and activation of rejection responses by their hosts, individual genets of these fungi are able specifically to parasitize and then take over the domain of whole populations of genets of *Coriolus* species (in the case of *L. betulina*) or *Bjerkandera* species (in the case of *P. gibbosa*) which had pioneered the colonization process.

CONCLUSIONS

We hope to have demonstrated during the course of this overview, some of the new ideas which are emerging as a consequence of

a deeper understanding of the versatility and responsiveness of the vegetative thalli of decomposer basidiomycetes. There is much to be substantiated and almost unlimited scope for further exploration. Above all, here is a field where the fusion of ecological, plant pathological, developmental, genetic and molecular approaches is not only a desirable but also a realistic prospect. Thereby may be provided an example for biologists in general.

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